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THE BIOLOGICAL BULLETIN

PUBLISHED BY
THE MARINE BIOLOGICAL LABORATORY

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1930–1947

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June 27, 1870 – November 5, 1947
Managing Editor
1902–1926
Member of the Editorial Board
1927–1947

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THE BIOLOGICAL BULLETIN

PUBLISHED BY THE MARINE BIOLOGICAL LABORATORY

THE MARINE BIOLOGICAL LABORATORY

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II. ACT OF INCORPORATION

No. 3170

COMMONWEALTH OF MASSACHUSETTS

Be It Known, That whereas Alpheus Hyatt, William Sanford Stevens, William T. Sedgwick, Edward G. Gardiner, Susan Minns, Charles Sedgwick Minot, Samuel Wells, William G. Farlow, Anna D. Phillips, and B. H. Van Vleck have associated themselves with the intention of forming a Corporation under the name of the Marine Biological Laboratory, for the purpose of establishing and maintaining a laboratory or station for scientific study and investigation, and a school for instruction in biology and natural history, and have complied with the provisions of the statutes of this Commonwealth in such case made and provided, as appears from the certificate of the President, Treasurer, and Trustees of said Corporation, duly approved by the Commissioner of Corporations, and recorded in this office;

Now, therefore, I, HENRY B. PIERCE, Secretary of the Commonwealth of Massachusetts, *do hereby certify* that said A. Hyatt, W. S. Stevens, W. T. Sedgwick, E. G. Gardiner, S. Minns, C. S. Minot, S. Wells, W. G. Farlow, A. D. Phillips, and B. H. Van Vleck, their associates and successors, are legally organized and established as, and are hereby made, an existing Corporation, under the name of the MARINE BIOLOGICAL LABORATORY, with the powers, rights, and privileges, and subject to the limitations, duties, and restrictions, which by law appertain thereto.

Witness my official signature hereunto subscribed, and the seal of the Commonwealth of Massachusetts hereunto affixed, this twentieth day of March, in the year of our Lord One Thousand Eight Hundred and Eighty-Eight.

[SEAL]

HENRY B. PIERCE,
Secretary of the Commonwealth.

III. BY-LAWS OF THE CORPORATION OF THE MARINE BIOLOGICAL LABORATORY

I. The members of the Corporation shall consist of persons elected by the Board of Trustees.

II. The officers of the Corporation shall consist of a President, Vice President, Director, Treasurer, and Clerk.

III. The Annual Meeting of the members shall be held on the second Tuesday in August in each year, at the Laboratory in Woods Hole, Massachusetts, at 11:30 A.M., and at such meeting the members shall choose by ballot a Treasurer and a Clerk to serve one year, and eight Trustees to serve four years, and shall transact such other business as may properly come before the meeting. Special meetings of the members may be called by the Trustees to be held at such time and place as may be designated.

IV. Twenty-five members shall constitute a quorum at any meeting.

V. Any member in good standing may vote at any meeting, either in person or by proxy duly executed.

VI. Inasmuch as the time and place of the Annual Meeting of members are fixed by these By-laws, no notice of the Annual Meeting need be given. Notice of any special meeting of members, however, shall be given by the Clerk by mailing notice of the time and place and purpose of such meeting, at least fifteen (15) days before such meeting, to each member at his or her address as shown on the records of the Corporation.

VII. The Annual Meeting of the Trustees shall be held on the second Tuesday in August in each year, at the Laboratory in Woods Hole, Mass., at 10 A.M. Special meetings of the Trustees shall be called by the President, or by any seven Trustees, to be held at such time and place as may be designated, and the Secretary shall give notice thereof by written or printed notice, mailed to each Trustee at his address as shown on the records of the Corporation, at least one (1) week before the meeting. At such special meeting only matters stated in the notice shall be considered. Seven Trustees of those eligible to vote shall constitute a quorum for the transaction of business at any meeting.

VIII. There shall be three groups of Trustees:

(A) Thirty-two Trustees chosen by the Corporation, divided into four classes, each to serve four years; and in addition there shall be two groups of Trustees as follows:

IV. THE REPORT OF THE TREASURER

To the Trustees of the Marine Biological Laboratory:

Gentlemen:

Herewith is my report as Treasurer of the Marine Biological Laboratory for the year 1946.

The accounts have been audited as in recent years by Messrs. Seamans, Stetson, and Tuttle, certified public accountants of Boston. A copy of their report is on file at the Laboratory office and inspection of it by Trustees or other Members of the Corporation will be welcomed.

The principal summaries of their report—The Balance Sheet, Statement of Income and Expense, and Current Surplus Account—are appended hereto as Exhibits A, B, and C.

The financial operations of 1946 may be summarized as follows:

I. Assets

1. Endowment Assets

As of December 31, 1946, the total book value of all the Endowment Assets, including the Scholarship Funds, was $977,199.30, an increase for the year of $10,427.14 due to profits on securities sold.

These Assets consisted of: marketable securities (bonds, preferred stocks, and common stocks) with a book or cost value of $889,416.18 and a market value of $934,042.19; six participations in mortgages on New York City realty costing $82,491.14, estimated by the Treasurer to have an actual value of $52,000,000; and $5,291.98 in uninvested principal cash.

The total current valuation of Endowment Assets was $991,334.17, $14,134.87 more than current book value, but $13,603.44 less than the actual value at the end of the previous year. This slight decline in market values during the year was due chiefly to lower prices for some of the common stocks.

2. Plant Assets

The total of Plant Assets at the end of the year was $1,312,963.70, or $17,884.05 less than at the end of 1945. New expenditures were more than offset by the depreciation charge of $25,314.41. In addition to heavy expenditures for repairs that were regarded as current expenditures, a total of $21,425.02 was paid out for improvements to buildings and new equipment that were regarded as capital items. To take care of some of these payments $15,000 of the Reserve Fund was used, leaving a balance in the Reserve Fund of $15,859.89, a substantial portion of which has already been appropriated for repairs and equipment.

3. Current Assets

At the end of the year Current Assets had increased by $7,881.53 to a total of $220,851.88. Accounts Payable were $8,411.64. Current Surplus had increased $6,022.47 to $202,360.31.

II. Income and Expenditures

Both income and expense totals were much larger than in recent years. Total Income was $227,104.26. Total Expense, including $25,314.41 Reserve for Depreciation, was $234,537.13, leaving an accounting deficit of $7,432.87 compared with a surplus of $9,773.28 for 1945. The 1946 expenditures contained a number of unusual items such as $11,510.00 for "Special Repairs to the Brick Laboratory" and further "1944 Hurricane Damage Repairs" of $2,012.50.

On December 31, 1946, current cash on hand amounted to $28,135.30, but as in the case of the Reserve Fund, a portion of this was designated for further improvements and purchases already authorized.

Noteworthy in the income account are the contributions totalling $1,070.00 from the Marine Biological Laboratory Associates that were used for purchases of especially needed apparatus and a gift of $700 from Mrs. W. Murray Crane.

The total receipts of the Supply Department also attained what is believed to be a new high, $73,394.26. The expenses of the Supply Department, particularly the purchases of specimens from outside sources, also increased however to $62,527.53, so that the net gain from operations rose only moderately to $10,866.73.

EXHIBIT A

Marine Biological Laboratory Balance Sheet, December 31, 1946

Assets

Endowment Assets and Equities:			
Securities and Cash in Hands of Central Hanover Bank and Trust Company, New York, Trustee		$ 960,062.58	
Securities and Cash in Minor Funds		17,136.72	
			$ 977,199.30
Plant Assets:			
Land	$ 110,425.38		
Buildings	1,324,036.67		
Equipment	193,539.92		
Library	349,234.22		
	$1,977,236.19		
Less Reserve for Depreciation	696,981.38		
		$1,280,254.81	
Reserve Fund, Securities and Cash		15,859.89	
Book Fund, Securities and Cash		16,849.00	
			$1,312,963.70

Current Assets:			
Cash		$ 28,135.30	
Mortgage Note Receivable		2,500.00	
Accounts Receivable		30,114.98	
Inventories:			
Supply Department	$ 39,083.91		
Biological Bulletin	18,557.44	57,641.35	
Investments:			
Devil's Lane Property and Accounts Receivable	$ 48,055.31		
Gansett Property and Accounts Receivable	1,074.67		
Stock in General Biological Supply House, Inc.	12,700.00		
Other Investment Securities	20,795.00		
Retirement Fund Securities and Cash	12,757.24	95,382.22	
Prepaid Insurance		4,556.59	
Items in Suspense (Debits)		2,521.44	
			$ 220,851.88
Total Assets			$2,511,014.88

Liabilities

Endowment Funds:			
Endowment Funds	$ 958,871.15		
Reserve for Amortization of Bond Premiums	1,191.43		
		$ 960,062.58	
Minor Funds		17,136.72	
			$ 977,199.30
Plant Funds:			
Mortgage Note Payable		$ 5,000.00	
Donations and Gifts	$1,172,564.04		
Other Investments in Plant from Gifts and Current Funds	135,399.66	1,307,963.70	
			$1,312,963.70
Current Liabilities and Surplus:			
Accounts Payable		$ 8,411.64	
Items in Suspense (Credits)		$ 1,751.24	
Reserve for Repairs and Replacements		8,328.63	
Current Surplus		202,360.37	
			$ 220,851.88
Total Liabilities			$2,511,014.88

EXHIBIT B

Marine Biological Laboratory Income and Expense, Year Ended December 31, 1946

	Total		Net	
	Expense	Income	Expense	Income
Income:				
General Endowment Fund		$ 29,966.14		$ 29,966.14
Library Fund		6,000.47		6,000.47
Donations		2,170.00		2,170.00
Instruction	$ 11,788.90	9,030.00	$ 2,758.90	
Research	6,329.17	20,645.14		14,315.97
Evening Lectures	75.00		75.00	
Biological Bulletin and Membership Dues	10,500.17	12,863.15		2,362.98
Supply Department	62,527.53	73,394.26		10,866.73
Mess	32,567.63	30,069.72	2,497.91	
Dormitories	30,640.35	16,880.30	13,760.05	
(Interest and Depreciation charged to above 3 Departments)	(26,008.91)			26,008.91
Dividends, General Biological Supply House		17,272.00		17,272.00
Dividends, Other Investment Stocks		1,000.00		1,000.00
Rents:				
Bar Neck Property	873.71	6,000.00		5,126.29
Janitor House	21.45	360.00		338.55
Sale of Library Duplicates, Micro Film, etc.		331.61		331.61
Microscope and Apparatus Rental		1,011.47		1,011.47
Sundry Income		10.00		10.00
Interest on Mortgage Receivable		100.00		100.00
Maintenance of Plant:				
Buildings and Grounds	28,337.37		28,337.37	
Apparatus Department	8,267.02		8,267.02	
Chemical Department	3,859.74		3,859.74	
Library Expense	6,664.76		6,664.76	
Workmen's Compensation Insurance	435.15		435.15	
Truck Expense	157.11		157.11	
Bay Shore Property	172.32		172.32	
Great Cedar Swamp	22.80		22.80	
General Expenses:				
Administration Expense	16,429.92		16,429.92	
Endowment Fund Trustee and Safe-Keeping	1,033.70		1,033.70	
Bad Debts	352.83		352.83	
Special Repairs 1944 Hurricane Damage	2,012.50		2,012.50	
Cost of Living Bonus	240.00		240.00	
Gift to Naples Biological Station	100.00		100.00	
Special Repairs, Brick Laboratory	11,510.00		11,510.00	
Interest	312.50		312.50	
Reserve for Depreciation	25,314.41		25,314.41	
	$234,537.13	$227,104.26	$124,313.99	$116,881.12
Excess of Expense over Income carried to Current Surplus		7,432.87		7,432.87
		$234,537.13		$124,313.99

EXHIBIT C

MARINE BIOLOGICAL LABORATORY CURRENT SURPLUS ACCOUNT, YEAR ENDED DECEMBER 31, 1946

Balance, January 1, 1946			$196,337.90
Add:			
Transfer from Reserve Fund		$15,000.00	
Reserve for Depreciation Charged to Plant Funds		25,314.41	40,314.41
			$236,652.31
Deduct:			
Excess of Expense over Income for Year		$ 7,432.87	
Payments from Current Funds during Year for Plant Assets:			
Buildings	$ 2,691.13		
Equipment	8,324.14		
Library	10,409.75		
	$21,425.02		
Less Received for Plant Assets Sold	3,306.90	18,118.12	
Transfers to Reserve Fund:			
Portion of Dividends from General Biological Supply House	$ 2,500.00		
Dividends from Crane Co.	875.00		
Gansett Property Profits, 1945	464.18	3,839.18	
Retirement Fund:			
Payments to Pensioners	$ 3,460.00		
Less Retirement Fund Income	200.11		
	$ 3,259.89		
Loss on Sale of Interest in Real Estate	1,030.34	4,290.23	
Loss on Sale of Gansett Property in 1946		81.60	
Adjustment of Sundry Accounts-Receivable, etc., account of Prior Years		529.94	34,291.94
Balance, December 31, 1946			$202,360.37

Respectfully submitted,

DONALD M. BRODIE,
Treasurer

V. REPORT OF THE LIBRARIAN

1946

The sum of $13,500 appropriated to the library in 1946 was expended as follows: books, $546.47; serials, $2,747.01; binding, $618.39; express, $74.34; supplies, $431.05; salaries, $7,886.13 ($1,700 of this sum was contributed by the Woods Hole Oceanographic Institution); back sets, $417.04; insurance, $45.00; total, $12,765.43. The cash receipts of the library totalled $331.61: for microfilms, $195.03 ($78.45 expenses paid by the library and accounted above under "supplies"); sale of duplicates, $135.65; sale of the "Serial List" Biological Bulletin supplement number, $0.93. This sum, $331.61, reverts to the laboratory and does not include rent payments for library readers which are collected by the main office. There were 38 library readers accommodated in the library during the summer of 1946, and practically all of these for the whole season, an improvement over 1945 when many of the 49 readers rented desks for short terms.

Of the Carnegie of New York Fund, $1,534.46 was expended for the completion of four back sets and for the partial completion of one other.

The sum appropriated by the Woods Hole Oceanographic Institution in 1946 for purchases was $800. A balance of $802.27 remaining from 1945 made an available total of $1,602.27. The expenditures amounted to $1,910.43, overrunning the account by the sum of $308.16. In addition to the above, the Woods Hole Oceanographic Institution contributed $1,700 (see above under salaries).

During 1946 the library received 1,065 current journals: 320 (19 new) by subscription to the Marine Biological Laboratory; 43 (15 new) to the Woods Hole Oceanographic Institution; exchanges with the "Biological Bulletin" 433 (13 new; 68 reinstated foreign) and 96 (21 new; 17 reinstated foreign) with the Woods Hole Oceanographic Institution; 165 as gifts to the former and 8 to the latter. The Library acquired 261 books: 81 by purchase of the Marine Biological Laboratory; 53 by purchase of the Woods Hole Oceanographic Institution; 13 gifts by the authors; 22 by the publishers; 48 by miscellaneous donors; 23 from the French Embassy Library, New York City; 1 from Dr. R. S. Lillie; 3 from Dr. Oliver S. Strong; 1 from Dr. Alfred C. Redfield; and 16 from Mrs. Priscilla B. Montgomery. There were 43 back sets of serial publications completed; 15 purchased by the Marine Biological Laboratory (4 with the Carnegie Fund); 7 by the Woods Hole Oceanographic Institution; 5 by exchange of the "Biological Bulletin"; 14 by exchange with the Woods Hole Oceanographic Institution publications; and 2 by gift and exchange of duplicate material. Partially completed sets were 73: purchased by the Marine Biological Laboratory, 10 (1 with the Carnegie Fund); purchased by the Woods Hole Oceanographic Institution, 10; by exchange with the "Biological Bulletin," 9; by exchange with the Woods Hole Oceanographic Institution publications, 11; by gift and exchange of duplicate material, 33.

The reprint additions to the library were 4,618: current of 1945, 841; current of 1946, 97; and of previous dates, 3,680. There were gifts of reprints from Dr. Alfred C. Redfield; Dr. E. G. Butler; and from Dr. L. C. Wyman.

At the end of the year 1946 the library contained 55,312 bound volumes and 142,292 reprints.

It is discouraging to report that no part of the German journals that have been lacking since 1940 have come through. We have heard, however, by letter of February 28, 1947, from the Otto Harrassowitz Company that there is "in preparation" a box of "stored material" that "will leave Berlin in summer via the Library of Congress Mission Berlin." We are hoping to discover whether this means some volumes of our standing order of the war years. If nothing comes to us in this direct way or through other of the Library of Congress "Mission Berlin" shipments I understand that a plan is already under way to reprint such lacking volumes provided a sufficient number of libraries are interested to assure the expense of the undertaking.

VI. REPORT OF THE DIRECTOR

To the Trustees of the Marine Biological Laboratory

Gentlemen:

I submit herewith the report of the fifty-ninth session of the Marine Biological Laboratory for the year 1946.

The past year has seen a continued recovery from war-time conditions. Our attendance increased, especially in the groups of Beginning Investigators and students, in large part due to returning veterans; many foreign journals have begun to come regularly after a lapse of several years; our gross income (and also expense) exceeded that of any recent year; the budget for research equipment was greatly increased; a good start was made in the repair of our buildings; the Lalor Fellowships were established.

1. *Financial*

During the past 10 years notable changes have occurred in the net income from some of our regular sources of revenue. Decreases appear in the return from the Endowments, from Instruction, and from the Mess; increases are seen in Research fees and the Supply Department. Changes in the cost of running the various departments have been due to war conditions. The accompanying table gives only the more important items of the budget. Net incomes are shown, rather than gross incomes. For the Supply Department, the Dormitories, and the Mess, the amounts do not include depreciation and other bookkeeping charges.

Net Income

	Endowments	Dividends	Research	Instruction	Supply Dept.	Dorm.	Mess
1946	35,965	18,270	14,315	(2,760)	14,545	5,290	780
1944	33,010	17,295	3,675	(2,915)	11,780	875	790
1942	36,085	11,420	4,950	(2,065)	1,760	9,275	1,385
1940	44,140	18,940	15,070	(615)	5,030	7,080	2,120
1936	50,190	12,700	10,070	2,130	9,160	6,660	3,125

Expense

	Bldgs. and Grds.	App. and Chem.	Library	Spec. Repairs	Admin.	Pensions
1946	37,420	15,055	17,075	13,525	16,430	3,460
1944	32,675	6,135	12,315	2,465	15,275	3,460
1942	25,130	9,805	15,335	5,810	12,500	3,460
1940	28,785	19,005	17,780	1,650	12,425	3,710
1936	37,925	17,190	22,140	—	14,910	4,060

Most important is the decline in the return from the Endowments. In 1930 our receipts from these sources amounted to $57,000. A sharp drop occurred in 1933, and smaller but steady declines each year until 1946. Our income now is less than two-thirds of the 1930 amount. In the meantime, returns from Dividends have risen, and have made up nearly half of the loss incurred in the Endowment income. The budget for Instruction showed a substantial loss during the war years when attendance was light. With larger numbers of students and an increase in tuition from $75.00 to $100.00 the deficit should disappear. The net gain from the Mess has fallen materially. In view of the high cost of food and services we are fortunate to end each season without loss.

On the other hand, the net return from Research fees is now high, due in part to rentals received from the Oceanographic Institution which has occupied rooms throughout the year. The Supply Department has been exceedingly active. For 1946 the gross receipts were over $73,000, as compared with $43,000 ten years ago. However, the net income has not increased proportionately, for the cost of specimens purchased from dealers, of containers, chemicals, and other items, has kept down the profits. Salaries have been increased by more than 25 per cent.

The major categories of expense, shown on the lower part of the Table are now, with one exception, only a little above what they were in 1936. During the war we could not obtain many items of scientific equipment and so could not replace outworn apparatus. The budget for the Apparatus Department was, therefore, small. Now when equipment is available we can not buy all that we need because of its high cost. So, although we are spending as much as we did 10 years ago, we obtain far less. Our physical plant fell into disrepair, but is now gradually being put into sound condition. The cost of this work in 1946 is shown in the column Special Repairs.

We stand in need of additional funds to increase our endowments, to improve and enlarge our research facilities, to make further essential repairs in our buildings, to improve the working conditions of the Supply Department, and finally, to replace the wooden laboratories with a modern, well equipped building. The Executive Committee submitted to the Rockefeller Foundation a full statement of our needs with a request for funds to meet all or a part of them. The Foundation, in reply, recognized the importance of these projects but stated that because of its large contribution to the Peking Union Medical College, it could not at this time consider our request. In view of this, the Laboratory, should seek moderate contributions from a number of sources, thus broadening our base of support. One such source is our own membership which has been asked to contribute to a fund for a new collecting boat. The response has been encouraging, the donors expressing their hearty approval of the project.

2. *Buildings and Grounds*

All of the Laboratory buildings will be in active use during the summer of 1947. The Lecture Hall, which was stripped of its salt water and gas when the Navy occupied it, is now fully restored and will be filled with investigators. Rooms in the Supply Department will also be used for research.

Repairs and replacements have been made elsewhere. The sea wall is now rebuilt, the waterproofing of the Brick Building is finished. The experience of the past winter has proved that the operation was successful. The Dormitory is now undergoing the same treatment. In addition, many of its rooms, left unpainted since the building was erected more than 20 years ago, will be painted. A most desirable improvement is the partial painting of the Auditorium.

Room 106 in the Brick Building has been transformed into an Associates Room. The Trustees, at the Special Meeting in Boston, directed that this be done in response to the request of the Associates who want some place in the Laboratory where they can meet. These friends will constitute an increasingly important part of our membership.

A part of the Devil's Lane Tract is now open, and roads through it have been constructed. Thus far 8 lots have been purchased by members of the Corporation. Now that all lots in the Gansett tract are sold, this new area is the only Laboratory property which is available to investigators who want to solve the housing problem by owning their own homes.

3. *The Lalor Fellowships*

Through the generosity and cooperation of the Lalor Foundation this Laboratory, during the next five years, can offer to well prepared young investigators, a place to work, adequate equipment, and funds sufficient for their living and travelling expenses. The Fellows will work primarily in the fields of Biophysics, Biochemistry, and Physiological Chemistry, but other fields are not excluded. Dr. Metz who conducted the negotiations with Dr. Burdick of the Lalor Foundation is to be congratulated on their successful conclusion.

4. *Gifts*

The Laboratory acknowledges with sincere thanks gifts from the following donors:

The Associates: $1,070 for the purchase of special apparatus.
Mrs. Murray Crane: A $1,000 bond, the proceeds of which have been used to purchase special apparatus.
Dr. W. D. Curtis: $100 for carpenter shop equipment.
Dr. A. C. Redfield: $100 for trees and shrubs.
Mr. Laurence Saunders: $200 for the bathing beach raft.

5. *Losses by Death*

The Laboratory has lost by death, since the last meeting of the Trustees, five men, three of whom were closely associated with the institution for more than 50 years.

Prof. William B. Scott, member of the Corporation since 1890; elected Trustee in 1897; Emeritus, 1931.
Prof. Robert A. Harper, Corporation member since 1891; Trustee from 1911 until his retirement in 1926 when he became Emeritus.
Mr. George M. Gray, laboratory assistant in 1891; full time manager of the Supply Department from 1899 until his retirement in 1933.
Prof. Herbert S. Jennings, Trustee from 1905 to 1938, when he was elected Trustee Emeritus.
Prof. Samuel O. Mast, Trustee from 1936 to 1943; Trustee Emeritus until his death.

We honor the memory of these men who through many years greatly helped in the development of this Laboratory.

6. Election of Trustees

At the Meeting of the Corporation on August 13, 1946, the following trustees were elected:

Class of 1950

D. E. S. Brown
D. P. Costello
M. H. Jacobs
D. M. Marsland
A. K. Parpart
F. Schrader
H. B. Steinbach
B. H. Willier

Class of 1949

F. A. Brown, Jr., to replace W. C. Curtis retired

Trustee Emeritus

W. C. Curtis

7. There are appended as parts of this report

1. Memorials to Dr. T. H. Morgan and C. E. McClung.
2. The Staff.
3. Investigators and Students.
4. Tabular View of Attendance.
5. Subscribing and Cooperating Institutions.
6. Evening Lectures.
7. Shorter Scientific Papers (Seminars).
8. Members of the Corporation.

Respectfully submitted,

CHARLES PACKARD,
Director

1. MEMORIALS TO DECEASED TRUSTEES

Thomas Hunt Morgan, 1866–1945

by E. G. Conklin

In the death of T. H. Morgan at Pasadena, California, on December 4, 1945, the world of science lost one of its foremost students of Experimental Zoology and Genetics, and the Marine Biological Laboratory one of its earliest and most eminent members. He was born at Lexington, Kentucky, September 25, 1866, member of a family distinguished in American history for patriotic and military achievements. He graduated from the University of Kentucky in 1886 with the degree of B.S. and was given the M.S. Degree in 1887. In the summer of 1886 he was a student at the marine laboratory at Annisquam, Massachusetts, during the last session of that laboratory, which was the immediate predecessor of the Woods Hole Marine Biological Laboratory. Thus he was the last surviving personal link between the Annisquam and the Woods Hole Laboratories. The Annisquam Laboratory was not widely known, although it was the successor through its founder, Alpheus Hyatt, of the famous but short-lived laboratory established by Louis Agassiz at Penikese Island in 1872. It would be interesting to learn how Morgan, immediately after his undergraduate course, came to attend this last session of the Annisquam Laboratory, but the fact of his attendance is evidence of his early interest in marine biology.

In the fall of 1886 he entered the Johns Hopkins University as a graduate student in the department of biology taking morphology under W. K. Brooks as his major subject and physiology under H. Newell Martin as his minor. His superior qualities were soon recognized by his appointment as teaching assistant during his first two years and by the University fellowship in biology during his third year. He received the Ph.D. degree in 1890 and was at once appointed to the Adam Bruce post-doctoral fellowship for the following year, part of which he spent on research expeditions in the Bahamas and Jamaica.

On his return from the West Indies in 1891 he was appointed Associate Professor of Biology at Bryn Mawr College, in succession to Professor E. B. Wilson who had been called to Columbia University. Morgan remained at Bryn Mawr as Associate Professor and Professor until 1904, when he was appointed Professor of Experimental Zoology at Columbia, where he remained for 24 years, during which his research work in genetics received world-wide recognition and, together with that of his students and associates, led to many fundamental discoveries regarding the mechanism of heredity and sex. Finally in 1928 he accepted the position of Professor of Biology and Director of the Wm. G. Kerckhoff Laboratories of Biology at the California Institute at Pasadena, where he remained for the balance of his life.

From his first acquaintance with marine biology at Annisquam to the end of his life Morgan was closely associated with marine laboratories, especially during his summer vacations. In 1888 and 1889, while still a graduate student, he occupied the Johns Hopkins Table at the laboratory of the U. S. Fish Commission at Woods Hole. Those were the first two years of the M. B. L. and although he is not listed as a member of this new laboratory for those years, he was closely associated with it from its beginning, for relations between these two Woods Hole laboratories in those days of few workers were very intimate. In 1890 Morgan became an active member of the M. B. L., occupied a room in the laboratory, gave lectures in the Zoology course, and one of the evening lectures, and became a member of the Corporation. In 1899 he became a life member, in 1897 a trustee and in 1936 trustee emeritus. During this long official connection, extending over 55 years, he was always an active collaborator in the plans and work of the laboratory, and was one of its most productive and inspiring investigators. He was for many years a member of the Executive Committee as well as of other special committees and his judgment and cooperation had much to do in shaping policy. Although he believed that once the laboratory was well endowed it should abandon teaching and devote all its resources to research, he nevertheless cooperated in the past and present policy of combining both teaching and research. In his earlier years here he lectured in some of the courses, and throughout his connection with the laboratory he served as a member of the "Staff of Investigation" and supervised the work of many research students. His general lectures to the whole laboratory were many and notable summaries of his research work. Sixteen such "Evening Lectures" are listed in the Annual Reports of the Laboratory and many of these were published in the former series of *Biological Lectures* or in the later *Biological Bulletin.*

He was rarely absent from the sessions of this laboratory, except in 1894, 1895 and 1900 when he was at the Zoological Station in Naples. Even after his chief interests had turned from experiments on marine animals to the genetics of insects he continued to come to Woods Hole summers, transporting his material and continuing his work here. And after his removal to California he and Mrs. Morgan continued to make the long journey to Woods Hole every summer until prevented by war conditions in the past two years. In California he made frequent visits to the marine laboratory of the California Institute at Corona del Mar, which he had established, and his last work there was done last summer on "Normal and Abnormal Development of the Eggs of Ciona" and was published about a month after his death.

When he was a student at Johns Hopkins, morphology, which was his chief subject, consisted largely of observational rather than experimental work and was centered to a large extent on the embryology of various classes of animals with the primary purpose of determining their phylogeny and relationships. Morgan's early researches were of this sort. By the time he had taken his Ph.D. he had finished eight papers and in the two following years he published eight more on this general theme. After 1892 he published no papers that were not experimental or analytical. This sudden change in his interests was due in large part to associations formed and interests aroused at Woods Hole and at the Naples Zoological Station. His experiments at Woods Hole began on teleost and echinoderm eggs in 1893, and at Naples in 1894 he collaborated with Hans Driesch on the embryolgoy of ctenophores and began experiments on many other groups. Thereafter he was always interested in the factors controlling development and always had some work going on that subject even when he was more deeply involved in other projects. In all he published about sixty papers and three books on the experimental embryology of animals belonging to ten different phyla or classes. Another field in which he did an enormous amount of work is regeneration, on which he published about forty papers and two books, on some eight classes of animals.

About the time of his removal to Columbia University in 1904 he began work on general cytology and especially on the cytology of sex determination. This led to extensive studies on the Cytological basis of peculiar sex ratios in parthenogenesis and on non-typical Mendelian ratios in general. Here also may be classed his studies on sex-limited and sex-linked heredity together with the causes of secondary sexual characters, as well as his numerous papers on fertility and sterility, which continued intermittently until the end of his life. In this field he published some fifty papers and two books.

Finally in the general field of Mendelism, mutations, and the "constitution of the germ plasm," he published either individually or in collaboration with assistants, about one hundred and twenty-five papers and ten books. Other publications of a more general character, such as those on evolution, natural selection, reviews, and biographies, bring the total number of his publications, as listed in the bound volumes of his reprints in our library and in unbound separates since 1937, to three hundred and seventy separate titles, of which twenty-four are books, some of which are translations into the Dutch, German, Spanish and Russian languages.

This long list of Morgan's published work on a great variety of subjects throws light on many of his personal characteristics. His biological interests were very extensive and he was extraordinarily alert in picking up new leads in research. He worked with great intensity and rapidity and published his journal articles promptly, and sometimes without adequate caution; as a result he often made mistakes. His laboratory room was always open to callers, but unless their business was important he did not permit them to interrupt his work. He would go right on with what he was doing and would answer questions without looking up from his microscope or dishes. But he was always cordial and helpful and if the business were important he would give it prompt attention. In more leisurely moments he loved to joke and gossip and was gay and full of fun, but in the laboratory he usually kept right on with his work. On one occasion during the first World War I wandered into his room and said, "This War is getting terrible." Without looking up he replied, "What war?" And yet he was as anxious as anyone about the course of events. He dwelt in no "ivory tower," but just then he was most interested in counting flies. I think I have never known any other man who wasted so little time. His work was his life.

He could do good work with the most primitive accommodations and instruments, and he cared little for show and finesse. His work room and table and aquaria might be crowded and apparently disorderly but he usually knew just where to put his hand on what he wanted. His microscope slides and bottles and dishes might be crudely labelled but they served his purpose. In short, he wasted no time on needless techniques but went as directly as possible to the results which he sought.

He disliked organizational and committee work, which is often a great waste of time, but in really important matters he took an active and important part, as in the affairs of the Marine Biological Laboratory, the American support of the Naples Station, the founding of the Journal of Experimental Zoology, and many other institutional and professional matters. In a halting and confused situation no one could be more helpful than he, for with lightening-like rapidity he would isolate the main issues from minor ones and go straight to a logical conclusion. His judgment of men was especially sound and forthright and he did not allow it to be warped by personal sentiments. But he was genuinely kind and affectionate with family, friends, students and associates, and no one learned, except by chance, of his many kindnesses and contributions to persons and institutions. I forbear to say more on this subject out of respect to his well known wishes.

He received abundant recognition and honors from many universities and scientific societies in America and foreign countries; he was president of our leading biological and zoological societies as well as of the more inclusive American Association for the Advancement of Science and the more exclusive National Academy of Sciences; he was a foreign member of the Royal Society and the one and only American zoologist to receive the Nobel Award; but these honors never changed in any respect his sincere and modest behavior.

When he began his epoch-making work on *Drosophila* who could have predicted the amazing discoveries which would be revealed in the course of a few years! It had been customary among students of heredity, following Weismann, to speak of "the architecture of the germ plasm," but practically nothing was known of that architecture except its location in the germ cells. After the work of Morgan and his associates that architecture became a detailed plan with precise location of hereditary factors or genes in chromosome maps, and in turn these maps led to fundamental discoveries in the phenomena of heredity itself. In this work he had the invaluable assistance of students who became his assistants and associates, and his judgment of men was never shown better than in his selection of these associates. Together they made an extraordinary team, working in harmonious cooperation and in a spirit of great adventure. Morgan was the leader of this team and he received chief recognition and honor for their important discoveries but he always gave full credit to his associates and shared his honors with them. And now this great leader and discoverer is gone from among us but his work and inspiration will remain with us and with future generations.

Professor C. E. McClung

April 5, 1870 to January 17, 1946

by F. R. Lillie

It is difficult, and indeed impossible in a short space, to do justice to the many-sided life of McClung. Here we record our sorrow and sense of loss to our own institution and express our gratitude for the many services that he rendered us. A graduate in Pharmacology of the University of Kansas in 1892, he received his A.B. Degree there in 1896 and, after study elsewhere, his Ph.D. Degree in 1902. He served as head of the department of Zoology at the University of Kansas until he succeeded the lamented Thomas H. Montgomery at the University of Pennsylvania in 1912. He continued unbroken Montgomery's loyalty and devotion to the M. B. L. and was elected a member of the Corporation and Trustee in 1913. With the exception of the year 1934 when he was visiting professor at Keio University in Tokyo, his membership was continuous until his death.

His first appearance at this Laboratory was as investigator and instructor in Embryology in 1903. Thereafter he was occupied in the work of the University of Kansas until his appointment at the University of Pennsylvania. He was then appointed on the M. B. L. staff of investigations and served throughout his life assisting not only his own students but many others who sought his advice. He also became interested in the library and served as Chairman of the Library Committee from 1923 to 1933 during the great development of the library after the erection of the main building in 1924; he was especially helpful when the General Education Board in 1925 presented $50,000.00 to be expended over a period of five years for purchase of books, periodicals and pamphlets, and in 1929 $200,000.00 for endowment of the library.

After the erection of the Crane Laboratory in 1913, the Laboratory rapidly became over-crowded again, and in 1919 the trustees decided that steps should be taken to provide construction and endowment that should be adequate to meet the requirements of a generation of biologists. One of the first steps toward this program was to approach the National Research Council through Dr. McClung, Chairman of the Division of Biology and Agriculture. He was instrumental in the appointment of a committee of his division to report on the situation. Their favorable report was approved by the Executive Board of the Council. This encouraged the presentation of the matter to the Rockefeller Foundation, and the raising of the funds for the great plan was soon well under way. Dr. McClung was also helpful at the laboratory from the start of the new plans. The arrangement of the large lecture room, which has proved so successful, was his suggestion.

Many of us remember Dr. McClung's kindliness, simplicity of character and friendliness. These qualities were displayed in many way in the social life of Woods Hole; thus, he served as President of the M. B. L. Tennis Club and also of the

M. B. L. Club, the social club of the students and investigators. Such activities, however, never detracted from his main interests in research, and a significant part of his most famous investigations on chromosomes was carried on here.

He was a member of the National Academy of Sciences, of the American Philosophical Society and of numerous national biological societies, and in most of them occupied positions of trust and influence at various times. He was indeed a consecrated man of science! His memory will long endure.

2. The Staff, 1946

Charles Packard, Director, Marine Biological Laboratory, Woods Hole, Massachusetts.

Senior Staff of Investigation

E. G. Conklin, Professor of Zoology, Emeritus, Princeton University.
Frank R. Lillie, Professor of Embryology, Emeritus, The University of Chicago.
Ralph S. Lillie, Professor of General Physiology, Emeritus, The University of Chicago.
S. O. Mast, Professor of Zoology, Emeritus, Johns Hopkins University.
A. P. Mathews, Professor of Biochemistry, Emeritus, University of Cincinnati.
G. H. Parker, Professor of Zoology, Emeritus, Harvard University.

ZOOLOGY

I. Consultants

T. H. Bissonnette, Professor of Biology, Trinity College.
L. L. Woodruff, Professor of Protozoology, Yale University.

II. Instructors

F. A. Brown, Associate Professor of Zoology, Northwestern University, in charge of course.
T. H. Bullock, Assistant Professor of Neurology University of Missouri Medical School.
W. D. Burbanck, Associate Professor of Biology, Drury College.
C. G. Goodchild, Professor of Biology, Southwest Missouri State Teachers College.
John H. Lochhead, Instructor in Zoology, University of Vermont.
Madelene E. Pierce, Assistant Professor of Zoology, Vassar College.
W. M. Reid, Assistant Professor of Biology, Monmouth College.
Mary D. Rogick, Professor of Biology, College of New Rochelle.

III. Laboratory Assistant

Antoin Baca, Duke University Medical School.

EMBRYOLOGY

I. Consultants

H. B. Goodrich, Professor of Biology, Wesleyan University.

II. Instructors

Donald P. Costello, Professor of Zoology, University of North Carolina, in charge of course.
Howard L. Hamilton, Assistant Professor of Zoology, Iowa State College.

John A. Moore, Assistant Professor of Zoology, Barnard College.
Albert Tyler, Assistant Professor of Embryology, California Institute of Technology.

III. Research Assistant

Marjorie Hopkins Fox, University of California.

IV. Laboratory Assistants

Catherine Henley, The Johns Hopkins University.
Eleanor Lerner, Washington University.

PHYSIOLOGY

I. Consultants

William R. Amberson, Professor of Physiology, University of Maryland, School of Medicine.
Harold C. Bradley, Professor of Physiological Chemistry, University of Wisconsin.
Walter E. Garrey, Professor of Physiology, Vanderbilt University Medical School.
Merkel H. Jacobs, Professor of Physiology, University of Pennsylvania.

II. Instructors

Arthur K. Parpart, Associate Professor of Biology, Princeton Universtiy, in charge of course.
E. S. Guzman Barron, Associate Professor of Biochemistry, The University of Chicago.
Edward Chambers, New York City.
Aurin M. Chase, Assistant Professor of Biology, Princeton University.
Arthur C. Giese, Associate Professor of Biology, Stanford University.
Rudolph T. Kempton, Professor of Zoology, Vasser College (absent in 1946).
Robert Ramsey, Associate Professor of Physiology, Medical College of Virginia.

BOTANY

I. Consultants

S. C. Brooks, Professor of Zoology, University of California.

II. Instructors

Maxwell S. Doty, Instructor of Botany, Northwestern University.
Hannah Croasdale, Dartmouth College.

EXPERIMENTAL RADIOLOGY

G. Failla, College of Physicians and Surgeons, Columbia University.
L. Robinson Hyde, Phillips Exeter Academy, Exeter, N. H.

LIBRARY

Priscilla B. Montgomery (Mrs. Thomas H. Montgomery, Jr.), Librarian
Deborah Lawrence, Assistant Librarian
Mrs. Elon Jessup Mary A. Rohan

APPARATUS DEPARTMENT

E. P. Little, Phillips Exeter Academy, Exeter, N. H., Manager

J. D. Graham
Dorothy LeFevre

CHEMICAL DEPARTMENT

E. P. Little, Phillips Exeter Academy, Exeter, N. H., Manager

SUPPLY DEPARTMENT

James McInnis, Manager
D. J. Zinn, Naturalist

Ruth Crowell
Grace M. Witzell
M. B. Gray
W. E. Kahler
F. N. Whitman
A. M. Hilton
G. Lehy

GENERAL OFFICE

F. M. MacNaught, Business Manager

Polly L. Crowell
Mrs. Lila S. Myers

GENERAL MAINTENANCE

T. E. Larkin, Superintendent

W. C. Hemenway
G. T. Nickelson, Jr.
R. W. Kahler
T. E. Tawell
A. J. Pierce

THE GEORGE M. GRAY MUSEUM

George M. Gray, Curator Emeritus

3. Investigators and Students

Independent Investigators, 1946

Abell, Richard G., Assistant Professor of Anatomy, University of Pennsylvania.
Addison, William H. F., Professor of Normal Histology and Embryology, University of Pennsylvania.
Anderson, Rubert S., Assistant Professor of Physiology, University of Maryland.
Anfinsen, Christian B., Instructor in Biological Chemistry, Harvard Medical School.
Armstrong, Philip B., Professor of Anatomy, College of Medicine, Syracuse University.
Ball, Eric G., Associate Professor of Biological Chemistry, Harvard Medical School.
Barron, E. S. Guzman, Associate Professor of Biochemistry, University of Chicago.
Bartlett, James H., Professor of Theoretical Physics, University of Illinois.
Berger, Charles A., Director, Biological Laboratory, Fordham University.
Bevelander, Gerrit, Associate Professor of Anatomy, New York University.
Bliss, Alfred F., Instructor in Physiology and Pharmacology, Albany Medical College.
Boell, Edgar J., Associate Professor of Biology, Yale University.
Bonner, John T., Junior Fellow, Harvard University.
Bridgman, Josephine, Associate Professor of Biology, Limestone College.
Brink, Frank, Jr., Research Associate, Johnson Foundation, University of Pennsylvania.
Brooks, Matilda M., Research Associate in Biology, University of California.
Brooks, Sumner C., Professor of Zoology, University of California.
Brown, Dugald E. S., Professor of Physiology, New York University.
Brown, Frank A., Jr., Associate Professor of Zoology, Northwestern University.

Brownell, Katherine A., Research Associate, Ohio State University.
Buck, John, National Institute of Health, Bethesda, Maryland.
Budington, Robert A., Professor of Zoology, Emeritus, Oberlin College.
Bullock, Theodore H., Assistant Professor of Anatomy, University of Missouri.
Burbanck, William D., Professor of Biology, Drury College.
Burkholder, Paul R., Professor of Botany, Yale University.
Cannan, R. Keith, Professor of Chemistry, New York University.
Chambers, Robert, Research Professor of Biology, New York University.
Chase, Aurin M., Assistant Professor of Biology, Princeton University.
Cheney, Ralph Holt, Chairman of Biology Department, Long Island University.
Chrystall, Frieda L., Teacher of Biology, Julia Richman High School.
Claff, C. Lloyd, Research Fellow in Surgery, Harvard Medical School.
Clark, Eleanor Linton, Voluntary Research Worker, University of Pennsylvania.
Clark, Eliot R., Professor of Anatomy, University of Pennsylvania School of Medicine.
Clement, A. C., Associate Professor of Biology, College of Charleston.
Clowes, G. H. A., Research Director, Emeritus, Eli Lilly and Company.
Cole, Kenneth S., Professor of Biophysics, University of Chicago.
Conklin, Edwin G., Professor of Biology, Emeritus, Princeton University.
Cooper, Kenneth W., Associate Professor of Biology, Princeton University.
Copeland, D. Eugene, Assistant Professor of Zoology, Brown University.
Copley, Alfred E., Research Associate, Mount Sinai Hospital.
Costello, Donald P., Professor of Zoology, University of North Carolina.
Croasdale, Hannah T., Technical Assistant, Dartmouth College.
Crouse, Helen V., Research Associate in Zoology, University of Pennsylvania.
Crowell, Sears, Associate Professor of Zoology, Miami University.
Curtis, W. C., Professor of Zoology, University of Missouri.
Dalcq, Albert M., Head of Anatomy and Embryology, Faculty of Medicine, Brussels.
Dalton, Howard C., Instructor in Biology, Brown University.
Dean, Helen W., Department of Anatomy, Harvard Medical School.
Diller, Irene Corey, Research Cytologist, Institute for Cancer Research, Lankinau Hospital.
Doty, Maxwell S., Instructor in Botany, Northwestern University.
Dumm, Mary E., Instructor in Chemistry, New York University Medical College.
Ellinger, Tage U. H., Professor of Zoology, Howard University.
Erdman, J. Gordon, Fellow, Mellon Institute of Industrial Research.
Failla, G., Professor of Radiology, Columbia University.
Figge, Frank H. J., Associate Professor of Anatomy, University of Maryland School of Medicine.
Fiske, Virginia Mayo, Instructor in Zoology, Wellesley College.
Frisch, John A., Professor of Biology, Canisius College.
Froehlich, Alfred, Associate, May Institute for Medical Research.
Furth, Jacob, Professor of Pathology, Cornell University Medical College.
Garrey, Walter E., Professor of Physiology, Emeritus, Vanderbilt University.
Giese, Arthur C., Associate Professor of Biology, Stanford University.
Gilbert, Perry W., Assistant Professor of Zoology, Cornell University.
Gilman, Lauren C., Assistant Professor of Zoology, University of South Dakota.
Glaser, Otto C., Professor of Biology, Amherst College.
Goodchild, Chauncey G., Professor of Biology, Missouri State College.
Gould, Harley N., Professor of Biology, H. Sophie Newcomb College.
Grand, C. G., Research Associate, New York University.
Gray, I. E., Professor of Zoology, Duke University.
Gregg, John R., Instructor in Biology, Johns Hopkins University.
Grundfest, Dr. Harry, Research Associate, Department of Neurology, Columbia University.
Harding, Clifford V., Assistant in Physiology, Brown University.
Harnly, Morris H., Associate Professor, Washington Square College.
Hartman, Frank A., Professor and Chairman, Department of Physiology, Ohio State University.
Harvey, Ethel Browne, Independent Investigator, Princeton University.
Harvey, E. Newton, Professor of Physiology, Princeton University.

Haywood, Charlotte, Professor of Physiology, Mount Holyoke College.
Heilbrunn, L. V., Professor of Zoology, University of Pennsylvania.
Hibbard, Hope, Professor of Zoology, Oberlin College.
Hopkins, Hoyt S., Associate Professor of Physiology, New York University.
Hunter, Francis Robert, Assistant Professor of Zoology, University of Oklahoma..
Hutchings, Lois M., Instructor in Biology, New York University.
Ifft, John D., Assistant Professor of Biology, Simmons College.
Jacobs, M. H., Professor of General Physiology, University of Pennsylvania.
Jakus, Marie A., Research Associate in Biology, Massachusetts Institute of Technology.
Jenkins, George B., Professor of Anatomy, Emeritus, George Washington University.
Jerome, Sister Francis, Instructor, Ohio State University.
Johlin, J. M., Associate Professor of Biochemistry, Vanderbilt Medical School.
Kaan, Helen W., Associate Professor of Zoology, Wellesley College.
Kemp, Margaret, Assistant Professor of Botany, Smith College.
Kemp, Norman E., Instructor in Biology, Wayne University.
Kindred, James E., Professor of Anatomy, University of Virginia.
Kleinholz, Lewis H., Guggenheim Fellow, Harvard University.
Klotz, John W., Instructor in Biology, Concordia Teachers College.
Kreezer, George L., Guggenheim Fellow, Princeton University.
Krugelis, Edith J., Instructor of Zoology, Vassar College.
Kuntz, Eloise, Assistant in Biology Department, Brown University.
Lavin, George I., In charge of Spectroscopic Laboratory, Rockefeller Institute for Medical Research.
Lazarow, Arnold, Instructor in Anatomy, Western Reserve Medical School.
LeFevre, Paul G., Instructor in Physiology, University of Vermont, College of Medicine.
Lillie, Ralph S., Professor of Physiology, Emeritus, The University of Chicago.
Lilly, Daniel M., Assistant Professor in Biology, Providence College.
Llana, Dr. Alfred H., Assistant Director, University of Chile.
Lochhead, John H., Assistant Professor of Zoology, University of Vermont.
Lucke, Balduin, Professor of Pathology, University of Pennsylvania School of Medicine.
Lynch, Rev. Wm. F., Teaching Fellow, New York University.
Manginelli, Piero, Assistant, Faculdade de Medicina, Sao Paulo, Brazil.
Marinelli, Leonides D., Physicist, Memorial Hospital.
Marmont, George H., Assistant Professor of Biophysics, University of Chicago.
Marsland, Douglas, Associate Professor of Biology, New York University.
Mast, S. O., Professor of Zoology, Emeritus, Johns Hopkins University.
Mathews, Albert P., Professor of Biochemistry, Emeritus, University of Cincinnati.
Mendes, Erasmo Garcia, Assistant Professor, University of Sao Paulo, Brazil.
Menkin, Valy, Assistant Professor of Pathology, Duke University School of Medicine.
Metz, Charles W., Director, Zoological Laboratory, University of Pennsylvania.
Moore, John A., Assistant Professor of Zoology, Barnard College.
Nachmansohn, David, Research Associate in Neurology, Columbia University.
Nelson, Leonard, Independent Investigator, University of Pennsylvania.
Northrop, John H., Member of the Institute, Rockefeller Institute for Medical Research.
Oppenheimer, Jane M., Assistant Professor of Biology, Bryn Mawr College.
Ormsbee, Richard A., Associate, Sloan-Kettering Institute, Memorial Hospital.
Orr, Paul R., Assistant Professor, Brooklyn College.
Oster, Robert H., Professor of Physiology, University of Maryland School of Medicine.
Osterhout, W. J. V., Member Emeritus, Rockefeller Institute for Medical Research.
Palade, George, Assistant Department of Anatomy, University of Bucharest, Rumania.
Parpart, Arthur K., Professor of Biology, Princeton University.
Pierce, Madelene E., Associate Professor of Zoology, Vassar College.
Pierson, Bernice F., Independent Investigator, Johns Hopkins University.
Plough, Harold H., Professor of Biology, Amherst College.
Poulson, D. F., Assistant Professor of Biology, Yale University.
Rakestraw, Norris W., Research Associate, Woods Hole Oceanographic Institute.
Ramsey, Robert W., Associate Professor of Physiology, Medical College of Virginia.
Rankin, John S., Assistant Professor of Zoology, University of Connecticut.

Reid, W. Malcolm, Head of Biology Department, Monmouth College.
Reinhard, Edward G., Professor of Biology and Head of Department, Catholic University of America.
Ris, Hans, Assistant in Physiology, Rockefeller Institute.
Robbie, Wilbur A., Research Associate, State University of Iowa.
Rogick, Mary Dora, Professor of Biology, College of New Rochelle.
Ryan, Francis, Assistant Professor of Zoology, Columbia University.
Schaeffer, A. A., Chairman Department of Biology, Temple University.
Scharrer, Ernst, Associate Professor of Anatomy, University of Colorado School of Medicine.
Schmidt, Gerhard, Senior Research Fellow in Medicine, Tufts College Medical School.
Schneyer, Leon H., Instructor, New York University Dental College.
Scott, Sister Florence Marie, Professor of Biology, Seton Hill College.
Shanes, Abraham M., Assistant Professor of Physiology, New York University College of Dentistry.
Shereshefsky, J. Leon, Professor of Physical Chemistry, Howard University.
Sichel, Dr. F., Professor of Physiology, University of Vermont College of Medicine.
Slifer, Eleanor H., Assistant Professor of Zoology, State University of Iowa.
Steinbach, H. B., Associate Professor of Zoology, Washington University.
Stewart, Dorothy R., Research Associate in Physiology, University of Pennsylvania Medical School.
Stokey, Alma G., Professor of Botany, Emeritus, Mount Holyoke College.
Stunkard, Horace H., Professor of Biology, New York University.
Sturtevant, Alfred H., Professor of Genetics, California Institute of Technology.
Taft, Charles H., Associate Professor of Pharmacology, University of Texas.
Taylor, Wm. Randolph, Professor of Botany, University of Michigan.
TeWinkel, Lois E., Associate Professor of Zoology, Smith College.
Thompson, Betty F., Instructor in Botany, Connecticut College for Women.
Todd, Edgar W., Visiting Investigator, Rockefeller Institute.
Tracy, Henry C., Professor of Anatomy, University of Kansas.
Tyler, Albert, Assistant Professor of Embryology, California Institute of Technology.
Villee, Claude A., Instructor in Biological Chemistry, Harvard Medical School.
Wainio, Walter W., Assistant Professor, New York University College of Dentistry.
Warner, Robert C., Assistant Professor of Chemistry, New York University College of Medicine.
Weber, Neal A., Associate Professor of Anatomy, University of North Dakota.
Weiss, Dr. Paul, Professor of Zoology, University of Chicago.
Wenrich, D. H., Professor of Zoology, University of Pennsylvania.
Whiting, Anna R., Instructor in Zoology, University of Pennsylvania.
Whiting, P. W., Associate Professor of Zoology, University of Pennsylvania.
Wichterman, Ralph, Assistant Professor of Biology, Temple University.
Wiercinski, Floyd J., Chairman and Professor of Biology, Lewis College of Science and Technology.
Wilber, Charles G., Instructor in Physiology, Fordham University.
Willier, B. H., Professor of Zoology, Johns Hopkins University.
Woodward, Arthur A., Jr., Research Assistant in Zoology, University of Pennsylvania.
Wrinch, Dorothy, Lecturer, Smith College.
Yntema, Chester L., Assistant Professor of Anatomy, Cornell University Medical College.

Beginning Investigators, 1946

Bender, Albert, Investigator, University of Pennsylvania.
Benedict, Dora, Student, Harvard Medical School.
Berg, George G., Graduate Student, Columbia University.
Blumenthal, Gertrude, University of Pennsylvania.
Brust, Manfred, Teaching Fellow in Biology, Washington Square College.
Chambers, Edward L., Research Associate, University of California.
Douglis, Marjorie B., Assistant in Zoology Department, University of Chicago.
Fisk, Albert A., Brown Fellow in Pathology, Yale University School of Medicine.

Goreau, Thomas F., Student, University of Pennsylvania School of Medicine.
Gould, David, Voluntary Investigator, New York University College of Medicine.
Green, James W., Graduate Student, Princeton University.
Greenberg, Ruven, Graduate Student, Ohio State University.
Gregory, John C., Graduate Student, Yale University.
Kozam, George, Graduate Student and Research Assistant, New York University Graduate School.
Lein, Joseph, Graduate Student, Princeton University.
Lovelace, Roberta, Teaching Fellow, University of North Carolina.
McLean, Dorothy J., Demonstrator, University of Toronto.
Moul, Edwin T., Teaching Fellow, University of Pennsylvania.
Narahara, Hiromichi, Columbia College of Physicians and Surgeons.
Recknagel, Richard O., Graduate Student, University of Pennsylvania.
Remond, A., Research Fellow, Johnson Foundation, University of Pennsylvania.
Roger, Henry C., Student, Deerfield Academy.
Roys, Chester C., University of Chicago.
Sheng, T. C., Graduate Student, Columbia University.
Taylor, Babette, Graduate Student, Washington University.
Weiner, Milton H., Student, Western Reserve University.
Weiss, Michael S., Teaching Fellow in Biology, New York University.
White, Elizabeth L., Graduate Student, Bryn Mawr College.
Wilson, T. Hastings, University of Pennsylvania Medical School.
Wilson, Walter L., Graduate Student, University of Pennsylvania.

Research Assistants, 1946

Abramsky, Tess A., Research Assistant, Rockefeller Institute for Medical Research.
Berman, Marjorie, Research Assistant, Columbia University.
Burt, Agnes S., Research Assistant, University of Chicago.
Campbell, Margaret L., Research Assistant, California Institute of Technology.
Carlson, Francis D., Research Assistant, Johnson Foundation, University of Pennsylvania.
DeFalco, Rose H., Research Assistant, University of Pennsylvania.
Dey, Thomas E., Research Assistant, Princeton University.
Dietz, Alma, Research Assistant, American International College.
Fox, Marjorie H., Research Fellow, University of California.
Hopkins, C. Nadine, Graduate Student, Northwestern University.
Kerschner, Jean, Research Assistant, University of Pennsylvania.
Lerner, Eleanor, Fellow in Zoology, Washington University.
Mitchell, Constance, Research Assistant, University of Pennsylvania.
Mitchell, Robert F., Research Assistant, New York University.
Norris, Karl H., Electronic Engineer, University of Chicago.
Rall, Wilfrid, Graduate Fellow in Biophysics, University of Chicago.
Robbie, Jean, Technical Assistant, Ohio State University.
Rosen, Mrs. David, Research Assistant, Yale University.
Rothenberg, Mortimer A., Research Assistant, College of Physicians and Surgeons, New York University.
Serin, B. Goldis, Research Assistant, University of Pennsylvania.
Ulled, Elias, Research Assistant, Temple University Medical School.
Vishniac, Wolf, Research Assistant, Washington University.
Wheeler, Charles B., Part time Instructor, University of Kansas.
Willis, Marian, Research Assistant, University of Pennsylvania.
Ziegler, Bernice, Laboratory Assistant, Washington University.

Library Readers, 1946

Amberson, William R., Professor of Physiology, University of Maryland School of Medicine.
Baker, Gladys E., Associate Professor of Plant Science, Vassar College.
Bennighof, C. L., Assistant Professor, Western Maryland College.

Bloch, Robert, Research Associate in Botany, Yale University.
Blum, Harold F., Guggenheim Fellow.
Bocher, Carol A., Research Assistant, The Johns Hopkins University.
Cassidy, Harold G., Assistant Professor of Chemistry, Yale University.
Cox, Edward H., Professor of Chemistry, Swarthmore College.
Dische, Zacharias, Visiting Scholar, College of Physicians and Surgeons, New York University.
Friedemann, Ulrich, Head of Department of Bacteriology, Jewish Hospital, Brooklyn.
Fries, E. F. B., Assistant Professor of Biology, City College of New York.
Gates, R. R., Professor of Botany Emeritus, University of London.
Grant, Madeline P., Member Science Faculty, Sarah Lawrence College.
Gudernatsch, Frederick, Visiting Professor, New York University.
Gurewich, Vladimir, Assistant Visiting Physician, Bellevue Hospital.
Hamilton, Howard L., Assistant Professor of Zoology, Iowa State College.
Keller, Rudolph, Researcher, The Robinson Foundation, Inc.
Krasnow, Frances, Head of Department of Research, Guggenheim Dental Foundation.
Loewi, Otto, Research Professor of Pharmacology, New York University College of Medicine.
Low, Eva M., Graduate Student, Yale University.
Lowenstein, Otto, Clinical Professor of Neurology, New York University College of Medicine.
McDonald, Sister Elizabeth Seton, Professor of Biology, College of Mt. Saint Joseph-on-the-Ohio.
Mavor, James Watt, Research Professor of Biology, Union College.
Meyerhof, Otto, Research Professor of Biochemistry, University of Pennsylvania.
Morris, Samuel
Moschcowitz, Eli, Assistant Professor Chemical Medicine, Columbia University.
Ponder, Eric, Research Investigator, The Nassau Hospital.
Price, Winston H., Research Assistant, Washington University Medical School.
Sandow, Alexander, Assistant Professor of Biology, New York University, Washington Square College.
Savage, Leonard J., Special Rockefeller Fellow, University of Chicago.
Schrank, A. R., Assistant Professor of Physiology, University of Texas.
Shwartzman, Gregory, Head of Department of Bacteriology, Mt. Sinai Hospital.
Speidel, Carl C., Professor of Anatomy, University of Virginia.
Stern, Kurt G., Adjunct Professor of Biochemistry, Polytechnic Institute of Brooklyn.
Straus, William L., Jr., Associate Professor of Anatomy, Johns Hopkins University.
White, Michael J. D., Reader in Zoology, University College, London.
Winsor, Charles P., Assistant Professor of Biology, Johns Hopkins School of Hygiene.
Zorzoli, Anita, Instructor in Physiology, Washington University School of Dentistry.

Students, 1946

BOTANY

Clifford, Sister Adele, S. C., Fordham University.
Fahey, Elizabeth Mary, Student, Boston University.
Fraas, Janet Lois, Student, Wheaton College.
Garnic, Justine, Student, Carnegie Institute of Technology.
Keeffe, Mary M., Student, Fordham University.
Torrey, Anna Marsh, Student, Swarthmore College.
Urquhart, Betty A., Instructor in Botany, Wheaton College, Wheaton, Illinois.
Vishniac, Wolf, Research Assistant, Washington University.
Wilson, Marie Ellen, Student, Western Maryland College.

EMBRYOLOGY

Banner, Albert H., Assistant Professor, University of Hawaii.
Barish, Natalie, Student, Goucher College.

Brinsley, Bertram, Graduate Student, New York University.
Bruch, Paul Robert, Student, Wesleyan University.
Bucklin, Donald Hartwell, 124 Woodbine Street, Providence, Rhode Island.
DiDea, Arthur, Assistant in Zoology, Washington University.
Ebert, James David, Student, Johns Hopkins University.
Eisenberg, Norma, Graduate Student, Brooklyn College.
Fitch, Naomi Selma, Student, McGill University.
Flood, Rev. Francis X., S. J., Assistant Professor of Biology, Canisius College.
Friedman, Florence L., Brooklyn College.
Galbreath, Jean L., Student Assistant, Elmira College.
Harrison, John W., Graduate Student, Washington and Jefferson College.
Jones, Alberta S., Student, Howard University.
Kuff, Edward Louis, Student, Johns Hopkins Medical School.
Leigh, Walter Henry, Instructor, Chicago City Junior College.
Liu, Chien-Kang, McGill University.
Markert, Clement Lawrence, Graduate Assistant, Johns Hopkins University.
Moore, Ellen Louise, Student, Wellesley College.
Morris, David Markland, Jr., Graduate Assistant and Student, Indiana University.
Mullally, Rev. Walter Bernard, Instructor in Biology, St. Anselm's College.
Murtland, Richard Lee, Graduate Student, Washington and Jefferson College.
Pequegnat, Willis Eugene, Assistant Professor of Zoology, Pomona College.
Schecter, David Edward, McGill University.
Seaman, Arlene R., Instructor in Zoology, Cornell University.
Shapiro, Esther May, Student, Goucher College.
Tatum, Anne, Student, Rosemont College.
Tiemeier, Otto W., Graduate Fellow, University of Illinois.
Tuttle, Ruth Frances, Instructor, Carnegie Institute of Technology.
Uber, Virginia Mae, Assistant in Biology, Pennsylvania College for Women.

PHYSIOLOGY

Abajian, John, Jr., Associate Professor of Anaesthesiology, University of Vermont.
Benedict, Dora, Student, Harvard Medical School.
Berg, George G., Graduate Student, Columbia University.
Clark, Carl Cyrus, Assistant, Columbia University.
Connelly, Clarence M., Graduate Student, Cancer Fellow, Johnson Foundation, University of Pennsylvania.
Crapster, Wirt Patterson, Student, Taneytown, Maryland.
Gregg, James H. Graduate Student, University of Miami.
Gregory, John C., Graduate Student, Yale University.
Guthe, Karl Frederick, Graduate Student, Harvard University.
Harting, Janey, Undergraduate Assistant, Washington University.
Hunter, Francis Robert, Assistant Professor, University of Oklahoma.
Joftes, David Lion, Graduate Student, Tufts College.
Johnson, Shirley Alma, Research Assistant, University of Toronto.
Kemp, Norman Everett, Instructor in Biology, Wayne University.
Levin, Ilene B., Goucher College.
Marshall, Jean McElroy, Instructor in Physiology, Mount Holyoke College.
McPhee, Gwelda S., Assistant in Physiology, Vassar College.
Mendes, Erasmo Garcia, Assistant Professor, University of Sao Paulo, Brazil.
Narahara, Hiromichi Tsuda, Columbia University.
Nelson, Thomas Clifford, Graduate Student, Assistant, Columbia University.
Roys, Chester C., University of Chicago.
Saslow, Herbert B., Laboratory Instructor and Graduate Student, University of Illinois.
Shulman, Nahum Raphael, Student, Johns Hopkins Medical School.
Silber, Earle, Student, University of Maryland Medical School.
St. George, Robert C. C., Jr., Graduate Student, Harvard University.
Umbarger, H. Edwin, 239 West Fifth Street, Mansfield, Ohio.

ZOOLOGY

Amberson, Margaret Mary, Oberlin College.
Banner, Albert Henry, Assistant Professor of Zoology, University of Hawaii.
Bergquist, Jeanne Bodin, Barnard College.
Bernsaw, Margaret Ida, Wilson College.
Bingham, Barbara Alice, University of Michigan.
Candelas, Gustavo, Assistant Instructor, University of Puerto Rico.
Cattell, Elly, Student, Cornell University.
Chadwick, John B., Harvard University.
Chivers, Mirian E., Howard University.
Denton, Winslow Crocker, Graduate assistant, Cornell University.
Edwards, John Parman, Student, Drury College.
Ehrentheil, Susanne Judith, Radcliffe College.
Emerson, Julia Ripley, Student, Wellesley College.
Enders, Abbie Gertrude, Student, Swarthmore College.
Feld, Emily Ann, Student, University of North Carolina.
Ferguson, Edward L., Student, Wesleyan University.
Foley, Joseph Brendan, Graduate student, Yale University.
Foreman, Darhl Lois, Student Assistant, George Washington University.
Fullerton, Ann Elizabeth, Student, Western Maryland College.
Gehr, Agnes Robbins, Student, Western Reserve University.
Gese, Edward Charles, Graduate Assistant, New York University.
Gese, Phyllis Kring, Student, New York University.
Hackett, Thomas Paul, 573 Purcell Avenue, Cincinnati, Ohio.
Hand, Cadet Hammond, Jr., Student, University of Connecticut.
Hopkins, Amos L., Jr., Student, Harvard College.
Hopp, William Beecher, Graduate student, Purdue University.
Humphrey, Judith M., Student, University of Connecticut.
Jakowska, Sophie, Fordham University.
Kenyon, V. Patricia, Graduate assistant, Brown University.
Kramer, David, Clark University.
Liberti, Alfred V., Graduate student, Fordham University.
Liu Chien-Kang, Graduate student, McGill University.
Machlis, Gertrude Rafferty, Assistant in Zoology, University of Illinois.
Meinkoth, Norman August, Assistant in Zoology, University of Illinois.
Mendes, Marta Vannucci, Assistant Professor, University of Sao Paulo.
Miller, Mary Elizabeth, Student, Western Maryland College.
Morris, David Markland, Jr., Graduate assistant in Zoology, Indiana University.
Moulton, James Malcolm, Undergraduate Laboratory assistant, Massachusetts State College.
Peters, Rev. W., Graduate student, Yale University.
Pollens, Norman Burton, Assistant in Zoology, University of Rochester.
Rice, Mary Esther, Drew University.
Robinson, Edwin James, Jr., Graduate assistant, New York University.
Sanderson, Margaret Crassons, Vassar College.
Saslow, Herbert B., Graduate assistant, University of Illinois.
Seitner, Philip G., Graduate Assistant, Purdue University.
Smith, Thomas C., Student, Oberlin College.
Sullivan, Rev. Thomas Donald, Fordham University.
Swanson, Ann Teresa, Graduate Assistant, Tufts College.
Thompson, Mary Jane, Graduate student, Yale University.
VanHoesen, Drusilla, Student, University of Pennsylvania.
Vivian, Janet, Graduate student, Radcliffe College.
Warner, Kathleen Louise, Instructor, Mundelein College.
Warters, Mary Ellen, Student, Oberlin College.
Wetmore, Katherine Beryl, Student, Radcliffe College.
Williams, Ernest E., Columbia University.

4. Tabular View of Attendance

	1942	*1943*	*1944*	*1945*	*1946*
Investigators—Total	201	160	193	212	267
Independent	132	89	112	138	175
Under instruction	16	19	11	10	29
Library readers	28	35	50	38	38
Research assistants	25	17	20	26	25
Students—Total	74	68	75	96	122
Zoology	36	47	37	55	57
Embryology	24	13	23	23	30
Physiology	6	8	10	13	26
Botany	8	—	5	5	9
Total Attendance	275	228	276	308	389
Less persons registered as both students and investigators	2	6	1		
	273	222	275		
Institutions Represented—Total	126	116	106	124	141
By investigators	83	70	74	100	102
By students	43	41	41	49	56
Schools and Academies Represented					
By investigators	2	2	1	2	2
By students	—	1	2	2	—
Foreign Institutions Represented					
By investigators	—	2	2	1	7
By students	—	—	3	—	5

5. Subscribing and Cooperating Institutions

1946

Cooperating Institutions

Amherst College
Barnard College
Bryn Mawr College
The Catholic University of America
Columbia University
Cornell University
Cornell University Medical College
Duke University
Fish and Wild Life Service, U. S. Department of the Interior
Fordham University
Goucher College
Harvard University
Harvard University Medical School
Johns Hopkins University
Lankenau Hospital Research Institute
Eli Lilly and Company
Massachusetts Institute of Technology
Mount Holyoke College
New York University
New York University College of Medicine
New York University School of Dentistry
New York University, Washington Square College
Oberlin College
Ohio State University
Princeton University
Rockefeller Institute for Medical Research
State University of Iowa
Syracuse University Medical School
Temple University
Tufts College
University of Chicago
University of Cincinnati
University of Illinois
University of Maryland Medical School
University of Missouri
University of Pennsylvania
University of Pennsylvania School of Medicine
University of Rochester
Vassar College
Washington University
Wellesley College
Wesleyan University
Western Reserve Medical School
Wheaton College
Wilson College
Woods Hole Oceanographic Institute
Yale University

Subscribing Institutions

American Philosophical Society (Penrose Fund)
Biological Institute, Philadelphia, Pennsylvania
Brown University
California Institute of Technology
Canisius College
Carnegie Institute of Technology
College of Mount Joseph-on-the-Ohio
Connecticut College for Women
Elmira College
Johns Hopkins Medical School
Lewis College of Science and Technology
Macy Foundation
Memorial Hospital
Miami University
Pomona College
Radcliffe College
St. Anselm's College
St. Michael's College
University of Kansas
University of Texas
University of Toronto
University of Vermont
Vanderbilt University Medical School
Western Maryland College

6. Friday Evening Lectures, 1946

Friday, June 28
Dr. Jane Oppenheimer .... "The Organization of the Teleost Blastoderm."

Friday, July 5
Prof. E. N. Harvey .... "The Effects of High Velocity Missiles on Tissue."

Friday, July 12
Dr. Benjamin W. Zweifach .... "The Relation of Metabolic Derangements of Liver and Kidney to Peripheral Vascular Reactions."

Friday, July 19
Dr. E. W. Dempsey .... "Observations on the Chemical Cytology of Several Mammalian Tissues."

Friday, July 26
Prof. K. S. Cole .... " "A" Bomb and Biology."

Friday, August 2
Dr. E. J. Cohn .... "The Separation of Plasma into Fractions Varying in Chemical and Biological Properties."

Friday, August 9
Dr. Eric G. Ball .... "Biochemical Studies on the Malarial Parasite."

Friday, August 16
Prof. M. H. Jacobs .... "Cell Membranes, Cell Permeability and Cell Volume."

Friday, August 23
Dr. E. J. Boell .... "Biochemical Differentiation during Amphibian Development."

Other Lectures

Thursday, July 18
Kodachrome Motion Picture
C. Lloyd Claff .... "Cardiac Resuscitation—Resuscitation Method after Asphyxia. Heart Defibrillation to Control Ventricular Fibrillation during Heart Surgery."

Thursday, July 25
Dr. Howard Meyerhof .... "The Crisis in Science Legislation."

Thursday, August 15
Prof. Harlow Shapley .... "Internationalism in Science."

Wednesday, August 21
Prof. J. H. Bartlett .... "A Possible Mechanism of Nerve Action."

Saturday, August 31
Dr. Paul S. Galtsoff .... "The Atom Bomb Test at Bikini."

7. Seminars, 1946

Tuesday, July 9
Dr. Albert Tyler .... "Inhibition of Fertilization in Sea Urchins by means of Univalent Antibodies vs. Antifertilizin.
Dr. W. A. Robbie .... "The Cyanide Sensitivity of the Unfertilized Sea Urchin Egg."
Dr. J. E. Kindred .... "The Effects of Mustard Vesicants on the Hemopoietic Organs."

Tuesday, July 16
Dr. A. F. Bliss .... "Intermediate Steps in the Visual Cycle."
Dr. A. M. Shanes .... "The Role of Metabolism in the Injury Potential of Frog Nerve."
Dr. W. H. Price .... "Adenosine Triphosphatase, Myosine, Actin, and their Relation to the Mechanico-Chemical Coupling of Muscle."

Tuesday, July 23
M. M. Brooks .... "Oxidation-reduction Studies as a Clue to the Mechanism of Fertilization of Marine Eggs."
C. L. Yntema .... "An Analysis of Induction of the Auditory Vesicle in the Salamander."
J T. Bonner .... "Aspects of Morphogenesis in the Slime Molds."

Tuesday, July 30
Dr. Ernst Scharrer ....,.... "Chemical Sense and Taste in the Sea Robin, Prionotus."
Dr. Claude A. Villee .... "Studies of the Respiration of the Imaginal Discs of Drosophila, using the Cartesian Diver Ultramicrorespirometer."
Dr. C. B. Anfinsen and .... "The Action of Naphthaquinone Antimalarials on Respiratory Enzymes."
Dr. E. G. Ball

Tuesday, August 6

Dr. A. R. Whiting
Dr. H. C. George .... "X-Ray Induced Visible and Dominant Lethal Mutations in Habrobracon Eggs."
Dr. P. B. Armstrong .... "Specificity of Cholin Esterase."
Dr. T. H. Bullock .... "The Giant Synapse in the Stellate Ganglion of the Squid as a Physiological-Preparation."

Dr. Berta Scharrer	"The Endocrine Function of the Corpora allata in Insects."

Tuesday, August 13

Dr. Katharine A. Brownell	"Evidence of a New Factor from the Adrenal."
Dr. F. A. Hartman	"Hyperactivity of the Adrenal Cortex."
Dr. Arnold Lazarow	"Studies on the Mechanism of Production of Diabetes with Alloxan."
Dr. Dorothy Wrinch	"On the Nature of Biological Specificity."

Tuesday, August 20

Dr. C. G. Wilber	"A Comparative Study of the Lipids in some Marine Annelids."
Dr. C. A. Berger	"Polyploid Mitosis as a Normal Factor in the Development of Allium cepa."
Dr. P. W. Whiting Dr. R. G. Schmieder	"Reproductive Economy in Close-crossed Species with Haploid Males."
Dr. T. U. H. Ellinger	"Hippocrates on Semen and on the Origin of the Child.

8. Members of the Corporation, 1946

1. Life Members

Allis, Mr. E. P., Jr., Palais Carnoles, Menton, France.
Beckwith, Dr. Cora J., Vassar College, Poughkeepsie, New York.
Billings, Mr. R. C., 66 Franklin Street, Boston, Massachusetts.
Calvert, Dr. Philip P., University of Pennsylvania, Philadelphia, Pennsylvania.
Cole, Dr. Leon J., College of Agriculture, Madison, Wisconsin.
Conklin, Prof. Edwin G., Princeton University, Princeton, New Jersey.
Cowdry, Dr. E. V., Washington University, St. Louis, Missouri.
Jackson, Mr. Chas. C., 24 Congress Street, Boston, Massachusetts.
Jackson, Miss M. C., 88 Marlboro Street, Boston, Massachusetts.
King, Mr. Chas. A.
Kingsbury, Prof. B. F., Cornell University, Ithaca, New York.
Lewis, Prof. W. H., Johns Hopkins University, Baltimore, Maryland.
Means, Dr. J. H., 15 Chestnut Street, Boston, Massachusetts.
Moore, Dr. George T., Missouri Botanical Gardens, St. Louis, Missouri.
Moore, Dr. J. Percy, University of Pennsylvania, Philadelphia, Pa.
Morgan, Mrs. T. H., Pasadena, California.
Noyes, Miss Eva J.
Porter, Dr. H. C., University of Pennsylvania, Philadelphia, Pennsylvania.
Scott, Dr. Ernest L., Columbia University, New York City, New York.
Sears, Dr. Henry F., 86 Beacon Street, Boston, Massachusetts.
Shedd, Mr. E. A.
Strong, Dr. O. S., Columbia University, New York City, New York.
Waite, Prof. F. C., 144 Locust Street, Dover, New Hampshire.
Wallace, Louise B., 359 Lytton Avenue, Palo Alto, California.

2. Regular Members

Adams, Dr. A. Elizabeth, Mount Holyoke College, South Hadley, Massachusetts.

Addison, Dr. W. H. F., University of Pennsylvania Medical School, Philadelphia, Pennsylvania.

Adolph, Dr. Edward F., University of Rochester Medical School, Rochester, New York.

Albaum, Dr. Harry G., Biology Dept., Brooklyn College, Brooklyn, N. Y.

Albert, Dr. Alexander, Mayo Clinic, Rochester, Minnesota.

Allee, Dr. W. C., The University of Chicago, Chicago, Illinois.

Amberson, Dr. William R., Department of Physiology, University of Maryland, School of Medicine.

Anderson, Dr. Rubert S., University of Maryland School of Medicine, Department of Physiology, Baltimore, Maryland.

Anderson, Dr. T. F., University of Pennsylvania, Philadelphia, Pennsylvania.

Armstrong, Dr. Philip B., College of Medicine, Syracuse University, Syracuse, New York.

Austin, Dr. Mary L., Wellesley College, Wellesley, Massachusetts.

Baitsell, Dr. George A., Yale University, New Haven, Connecticut.

Baker, Dr. H. B., Zoological Laboratory, University of Pennsylvania, Philadelphia, Pennsylvania.

Ballard, Dr. William W., Dartmouth College, Hanover, New Hampshire.

Ballentine, Dr. Robert, Columbia University, Department of Zoology, New York City, New York.

Ball, Dr. Eric G., Department of Biological Chemistry, Harvard University Medical School, Boston, Massachusetts.

Bard, Prof. Philip, Johns Hopkins Medical School, Baltimore, Maryland.

Barron, Dr. E. S. Guzman, Department of Medicine, The University of Chicago, Chicago, Illinois.

Barth, Dr. L. G., Department of Zoology, Columbia University, New York City, New York.

Bartlett, Dr. James H., Department of Physics, University of Illinois, Urbana, Illinois.

Beadle, Dr. G. W., School of Biological Sciences, Stanford University, California.

Beams, Dr. Harold W., Department of Zoology, State University of Iowa, Iowa City, Iowa.

Beck, Dr. L. V., Hahnemann Medical College, Philadelphia, Pennsylvania.

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THE PERIOD OF QUIESCENCE IN THE RESPONSE TO LIGHT BY AMOEBA

(THE RESPONSE TO LIGHT BY AMOEBA)

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INTRODUCTION

As is well known, an amoeba, when subjected to sudden illumination, responds by cessation of movement, and, as this cessation does not take place immediately, an interval exists between the increase in illumination and the reaction, thus giving rise to a reaction-time. As shown in a previous paper (Folger, 1925), the duration of the reaction-time depends on various factors, such as intensity of the light, previous stimulation, physiological condition of the animal. However, the reaction-time does not constitute the whole of the response to light by amoeba. After stopping, the animal remains motionless for some time, then resumes locomotion. The interval during which movement is suspended and which has been designated the quiescent period or period of quiescence is likewise dependent upon numerous factors and must necessarily be included in a complete investigation of the response to light by the organism.

Investigations dealing with a phase in the reaction of light similar to the one just mentioned have not been numerous. Possibly the growth reaction in a mold, as described by Blaauw (1914), may be compared to it. Blaauw was able by means of mirrors to illuminate the spore-carrier of *Phycomyces nitens* on four sides simultaneously, thus bringing about a symmetrical stimulation and avoiding bending. He found that after an illumination of one minute an increase in the rate of growth could be detected about three and a half minutes later, though this reaction-time was subject to much variation, depending both on the individual and on the intensity of the light. The increase in growth was augmented more and more until in some cases it amounted to as much as two hundred or even four hundred per cent of the normal rate. Shortly afterward, however, growth began gradually to slow down until finally it fell below normal, a condition which was of short duration for soon the plant was growing at its usual rate again.

Apparently more closely related to the phase to be considered in *Amoeba* is that encountered in streaming plant cells such as those of *Chara* and *Nitella,* as described by Ewart (1903), who gives an excellent review of previous work on protoplasmic streaming and adds the results of experiments by himself. Unfortunately for work with illumination, the cells of both of these plants contain chloroplasts, and photosynthesis brings in a complicating factor which interferes somewhat with a study of other effects of light. But in the case of several additional stimulating agents, especially mechanical shock, there is a response similar to that given by *Amoeba* to sudden illumination. The response of a streaming cell of *Chara* to mechanical shock, for instance, comprises a cessation followed shortly by a resumption of movement, and, as Ewart shows, a close relationship exists between the intensity of the stimulating agent and the time during which streaming remains suspended.

Some use has already been made of the period of quiescence in an investigation of the reactions of *Amoeba.* Folger (1926) demonstrated that in response to mechanical shock it becomes longer with increase in the magnitude of stimulation. This paper is concerned with a study of the quiescent period in the response of *Amoeba* to light.

MATERIALS AND METHODS

Apparatus similar to that used in the experiments to be presented has already been described (Folger, 1925). Light was obtained from a 1000-watt, 112-volt, cylindrical Mazda stereoptican lamp and flashed upon the amoebae by means of the plane mirror of the microscope. Heat rays were eliminated by placing a vessel of distilled water between lamp and mirror. The voltage of the current was about 114, and as the amoebae were placed at a distance of 25 centimeters from the light, they were, consequently, subjected to an illumination of approximately 16,000 meter candles intensity, even after some absorption of light by mirror and water. Active specimens of *Amoeba proteus* were used as experimental animals.

EXPERIMENTAL RESULTS

Mention has been made of the lengthening of the period of quiescence with increase in the magnitude of mechanical shock. In the experiments referred to, the shock was brought about by dropping pieces of wire through a glass tube to the slide on which the amoebae had been placed. Under these conditions the time of application of the stimulus did not vary, it might be described as instantaneous, and the distance through which the weights fell was not changed. Consequently the intensity of the shocks varied with the weights of the pieces of wire, which were all of the same diameter but of various lengths. In the experiments now to be described, the intensity of the stimulating agent, that is, the light, remained unchanged, but it was easy enough to vary the magnitude of the stimulus by altering the time of exposure. The following effects of light on the period of quiescence were investigated: (1) the effect of changing the length of exposure; (2) the effect of altering the time elapsing between exposures; (3) the effect of repeated exposures.

Relation between the period of quiescence and the exposure period

If one keeps the intensity of the light constant but alters the time during which it acts, it is immediately apparent that the quiescent period varies with the time of exposure. This is well illustrated by the results of the experiment recorded in Table 1, in which an amoeba was subjected to light for periods ranging from 4 seconds to 45 seconds. A response was obtained in every instance, with an average reaction-time of about 2.6 seconds.[1] As can be seen, it was quite otherwise with the period of quiescence. An exposure of 4 seconds produced a quiescent period of 10.6 seconds; one of 10 seconds, a quiescent period of 18.7 seconds; and one of 15 seconds, a quiescent period of 24 seconds. Thus the period of quiescence increased with increase of exposure and this continued until the exposure amounted to 45 seconds, when the period of quiescence was 42 seconds.

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[1] It is not to be assumed that length of exposure can have no effect on the reaction-time, but to produce a reaction-time essentially different from that given above would require a much shorter exposure than any used in this experiment.

Further analysis brings out other relationships between exposure and quiescent periods. In the final column is shown the difference obtained by subtracting the former from the latter. With an exposure of 4 seconds the difference amounted to 6.6 seconds. With an exposure of 10 seconds it was 8.7 seconds, and with an exposure of 15 seconds it was 9 seconds. Thus far the rate of increase has been greater in the period of quiescence than in the exposure period, but this did not continue indefinitely, for with an exposure of 20 seconds the difference had fallen to 7 seconds, with an exposure of 30 seconds it was 3 seconds, and with an exposure of 35 seconds it was 2 seconds. The exposure period is now increasing faster than the period of quiescence, and the ultimate result is shown at an exposure of 45 seconds, when the difference amounted to − 3 seconds. In other words, the exposure period has become longer than the period of quiescence. It has already been stated that the amoeba ceased to move after it was in illumination for about 2.6 seconds. So, when exposed for 45 seconds, it stopped in 2.6 seconds and resumed movement in another 42 seconds, 0.4 of a second before the light was turned off. Obviously an exposure greater than 45 seconds would not have called forth a longer period of quiescence, since the amoeba would still have stopped and then resumed movement within about 44.6 seconds after the light was turned on.

Table I

Showing increase of the period of quiescence with increase of exposure period. Time indicated by seconds.

Exposure period (s)	Number of tests	Period of quiescence (t)			t-s
		Maximum	Minimum	Average	
4	5	14	7	10.6	6.6
10	3	20	17	18.8	8.7
15	3	30	21	24.0	9.0
20	3	32	24	27.0	7.0
25	2	29	27	28.0	3.0
30	2	32	32	32.0	2.0
45	1			42.0	−3.0

Time required for recovery from the effects of sudden illumination

Reaction by *Amoeba* to light or to mechanical shock is, as previously stated, followed by a refractory period, which may be either absolute or relative; that is, the amoeba may refuse to react to a second stimulus, or it may react but with an altered response. Partial recovery from the effects of either kind of stimulus is indicated by a reaction-time that is longer than that obtained after complete recovery (Folger, 1925, 1926). The effect of partial recovery from sudden illumination on the period of quiescence is illustrated by the results of the experiment shown in Table II.

TABLE II

Showing the influence upon the period of quiescence on the lack of recovery from the effects of previous stimulation. Light turned on and left on until amoeba resumed locomotion.

Time in dark previous to exposure	Number of tests	Period of quiescence (sec.)		
		Maximum	Minimum	Average
4 seconds	4	No reaction		
10 seconds	4	30	18	22.5
30 seconds	6	40	15	24.8
1 minute	6	60	25	36.7
2 minutes	6	75	40	54.2
10 minutes	1			175.0
30 minutes	1			220.0
2 hours	1			218.0

In this experiment a single amoeba was used and was subjected to a series of tests in each of which it was first exposed to light for 1 minute, then to darkness for 3 minutes, and again to light, this time for 30 seconds. This procedure was intended to bring about a condition of uniformity. Following the 30-second exposures the light was turned off for intervals varying in the several trials from 4 seconds to 2 hours. Finally in each case the amoeba was then exposed to illumination and left exposed until the characteristic response was completed by the resumption of flow. As shown in the table, no reactions were obtained when 4 seconds were allowed for recovery. Ten seconds permitted a recovery sufficient to obtain reactions in every instance, with an average period of quiescence in 4 trials of 22.5 seconds. Thirty seconds for recovery resulted in an average period of quiescence of 24.5 seconds; 1 minute for recovery, in an average period of quiescence of 36.7 seconds; and 2 minutes for recovery, in an average period of quiescence of 54.2 seconds. Ten minutes, 30 minutes, and 2 hours for recovery were followed by periods of quiescence of 175 seconds, 220 seconds, and 218 seconds, respectively. Clearly the period of quiescence became longer as the amoeba recovered from the effects of previous stimulation, reaching a maximum when recovery was complete. Evidently under the conditions of the experiment complete recovery was not attained until after 10 minutes but before 30 minutes had elapsed. This contrasts with the 1 or 2 minutes necessary to obtain a minimum reaction-time (Folger, 1925, Alsup, 1937).

A few further words of explanation are necessary for a proper evaluation of the results just given. When an amoeba is exposed to light and ceases to move, the stoppage is generally sudden, permitting accurate timing with a stopwatch. If the exposure period is not too long or if too much time has not elapsed between stimuli, the resumption of movement also occurs with sufficient abruptness to be timed with a fair degree of precision. However, when a strong light is combined with sufficient time for complete or almost complete recovery from the effects of previous stimulation and with a long exposure period, expecially with one so long that the amoeba begins to move while the light is still acting, the resumption of flow cannot be ascertained with the same accuracy. Under these conditions the first indication of movement is usually an extrusion of a number of small pseudopodia, followed by an erratic flow, first in one direction, then in another. Gradually these pseudopodia lessen in number, movement becomes stronger, until finally the amoeba is again flowing briskly. If, now, one considers the first indication of movement as the end of the period of quiescence, it is easily determined. But if he assumes that this period is not completed until the amoeba has resumed the more vigorous movement that obtained before stimulation, he must exert a judgement, and the period can not be timed with the same degree of accuracy. In the present instance the period of quiescence was interpreted as ending when the protoplasmic flow became as strong as it was before stimulation, but even if the first indication of movement had been chosen as the final point in the reaction, while the higher figures in the preceding table would have been altered by several seconds, in the very long exposures even by as much as 25 or 30 seconds, the general aspect of a curve drawn from them would not be materially changed.

The effect of repeated stimulation

Repeated stimulation by light, with a short time intervening between exposures, brings out another peculiarity in the behavior of amoeba, which is exemplified by the results of the experiment summarized in Table III. This table records averages of the reactions of from 11 to 16 amoebae from each of 3 cultures. Each amoeba was subjected to darkness for at least 10 minutes and then given 10 successive 3-second exposures to light, with intervals of 1 minute in darkness between exposures. Though there were variations among the animals from the same culture and even more among animals from diverse cultures, the general behavior was the same in every case and is reflected in the total averages given in the table. The average period of quiescence for the first exposure amounted to 18.1 seconds, for the second exposure to 16.9 seconds, for the third exposure to 13.7 seconds. As one may see, this decline continued until the sixth exposure, when the period of quiescence was 10.3 seconds. In the 4 succeeding trials at no time did it fall far below 10 seconds

TABLE III

Showing decrease in period of quiescence with repeated exposures. Each amoeba subjected to darkness for at least 10 minutes and then given 10 successive 3-second exposures to light, with intervals of 1 minute in darkness between exposures. Number of animals in cultures 1, 2, 3, were 11, 13, and 16, respectively.

Reaction number	Average period of quiescence (sec.)			General average
	Culture No. 1	Culture No. 2	Culture No. 3	
1	18.5	21.6	14.3	18.1
2	17.8	18.6	14.4	16.9
3	12.6	14.0	14.6	13.7
4	11.6	14.3	12.6	12.8
5	10.4	12.4	12.4	11.7
6	8.8	11.9	10.1	10.3
7	10.8	11.5	11.9	11.4
8	10.4	9.4	10.8	10.2
9	8.8	8.2	9.9	9.0
10	10.3	10.1	8.8	9.7

and on the tenth exposure amounted to 9.7 seconds. Even when further tests were added, as they were in some instances though the results are not given in the table, there was no further reduction in the period of quiescence.

This diminution of the period of quiescence with repeated exposure is somewhat reminiscent of the phenomenon known as treppe, to be met with in the physiology of muscle.

DISCUSSION

The response to light that has been under consideration is intimately related to locomotion and must perhaps in final analysis be explained in terms of amoeboid movement. According to Mast (1926), an amoeba consists essentially of an inner fluid plasmasol and an outer solid plasmagel, which is surrounded by a very thin elastic surface membrane, the plasmalemma. The plasmasol is hypertonic and the plasmagel and plasmalemma act as semipermeable membranes, resulting in the development of an osmotic pressure in the plasmasol and a stretching of the plasmagel, especially at these points where it is weakest, where the pseudopodia are formed. Locomotion, according to Mast, involves a continuous change from plasmasol to plasmagel at the anterior end of the animal and from plasmagel to plasmasol at the posterior end. For further analysis of amoeboid movement one is referred to Mast's paper. For our purpose it will suffice to point out that when it is moving, the amoeba must be in a state of dynamic equilibrium. Illumination might cause an increase in the elastic strength of the plasmagel, especially at the tip of the advancing pseudopodium (Mast, 1932), bringing about a temporary breakdown in the established equilibrium, with consequent cessation of forward movement. Shortly an equilibrium is reformed and the animal again moves. Since experiments described in the preceding section have shown that stimulation applied shortly after the resumption of locomotion either fails to bring about a reaction or is followed by a reaction with a shortened period of quiescence, it is apparent that the new equilibrium is by no means at the same level as that which was in existence before the cessation occurred. Otherwise one would expect identical responses when the one stimulation is followed by another of equal strength. The experiments show, moreover, that the original level is gradually restored since a stimulus of a given intensity will finally elicit a second response of exactly the same magnitude as that which it called forth at first. Obviously, the time during which the disturbed equilibrium is reverting to the original level constitutes the refractory period.

SUMMARY

1. *Amoeba proteus* reacts to sudden illumination by cessation of movement. The time during which it is motionless has been designated the period of quiescence.

2. The period of quiescence becomes longer with extension of the time of exposure to illumination. At first the period of quiescence increases more rapidly than the exposure period, but soon the rate of increase of the latter becomes the greater, with the final result that the amoeba begins to move while the light is still on.

3. Stimulation by light is followed by a refractory period, which may be absolute

or relative; that is, the amoeba may either refuse to respond to a second stimulus, or it may react but with an altered response. Incomplete recovery from the effects of previous stimulation results in a period of quiescence that is shorter than that obtained after complete recovery.

4. If an amoeba is repeatedly exposed to illumination at intervals of a minute, the period of quiescence is at first relatively long, but becomes progressively shorter, arriving at a minimum after about 6 minutes.

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A METHOD FOR REMOVAL OF THE SINUS GLAND FROM THE EYESTALKS OF CRUSTACEANS[1]

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An increasingly large number of endocrine effects has been attributed to the crustacean sinus gland within the last decade (see reviews by Kleinholz, 1942 and by Brown, 1944). Efforts to determine the role played by these reported endocrine effects in the normal physiology of the animal have proceeded along conventional paths. Although investigators have applied the classical methods of endocrinology in determining in crustaceans the endocrine function of a suspected organ, i.e., deficiency and replacement experiments, the deficiency experiments have not been completely adequate; at times the uneasy feeling arises that some of the reported experimental results may be artifacts.

This situation has come about because removal of the sinus gland, located between the 2nd and 3rd optic ganglia of the eyestalk, is most simply accomplished by ablation of both eyestalks. As has already been pointed out (Kleinholz 1938, 1942), such surgery removes at the same time a not inconsiderable portion of the central nervous system, the four large ganglia located in each stalk. Control experiments to eyestalk ablation, in which antennae or other appendages are removed to simulate equivalent nerve injury or traumatic damage to the experimental animal, are thus not qualitatively comparable. The extent to which these ganglia in the eyestalk are centers of reflex activity, other than visual, has apparently not been adequately determined; the possibility exists that these ganglia of the eyestalk may function as inhibitory centers of nervous activity as does the brain itself (Bethe, 1898; Roeder, 1937).

In recognition of the fact that the most facile surgical procedure is not necessarily the most critical or advisable one, is the growing realization that deficiency experiments in the physiological study of the sinus gland must be made after removal of the sinus gland alone, with as little attendant injury to the nerve ganglia as is possible. Technically, this is not always feasible, especially among the smaller crustaceans. With the larger macrurans and brachyurans, however, surgical excision of the sinus gland is possible by the application of a little ingenuity. Brown (1942) has described such a method for removing the sinus gland from the eyestalk of the crayfish. The procedure described in this note allows removal of the sinus gland with perhaps less disturbance or injury to the eyestalk than the method of Brown. As described here, the method represents the adoption, over several years, of slight modifications in technique to solve the various difficulties that appeared during removal of the sinus gland; other investigators who may attempt such surgery will probably discover alternative improvements in the method.

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[1] This work was started in 1941 with the aid of a grant from the Permanent Science Fund of the American Academy of Arts and Sciences. Continuation after the war was made possible by a John Simon Guggenheim Memorial Fellowship. I am indebted to the Chairman of the Division and to the Director of the Biological Laboratories of Harvard University for the many courtesies and facilities extended me.

Such gland removal has been performed on the crayfish and the lobster, the latter animal being easier to operate upon because of its larger size. The steps outlined below apply more readily to the crayfish.

It was early found necessary to have the animal anesthetized, because with an animal that struggled during the more delicately manipulative steps of gland removal, serious damage of the nervous components of the eyestalk would result. Trial of several methods of anesthesia (chloretone, cold) were unsatisfactory; diluted chloroform water proved to be excellent for the purpose. This was prepared by saturating 100 ml. of water with 1 ml. of chloroform by vigorous shaking, allowing the mixture to stand for 2 or 3 minutes so that undissolved chloroform droplets would settle, and pouring the supernatant into a large beaker to which was added about 500 ml. of water. The crayfish was immersed in this dilute chloroform solution for 1–3 minutes; much longer immersion gave too deep anesthesia which was not always followed by successful recovery. After removal from the chloroform solution the animal was washed briefly in running tap water and fastened to an operating board.

The operating board was a rectangular piece of plywood, about 3½ inches by 6 inches in its dimensions, with a short stout elastic rubber band encircling each end. The anesthetized animal is held in place by inserting the chelipeds under the elastic band at one end, and the abdomen under the rubber band at the other end. After thus fastening the animal, a ligature of cotton thread is loosely tied around the non-skeletogenous base of the eyestalk. This serves as an effective hemostat during later steps of gland removal; excessive bleeding can be prevented or reduced by tightening the ligature. A small piece of modelling clay is used as a wedge between the stalk and the rostral orbit (Fig. 1); this keeps the eyestalk in a fixed position and prevents damage to the retina that might ordinarily result from pressure of the stalk against the carapace or rostral base during subsequent trephining.

Trephination of the exoskeleton is accomplished with the aid of drills of appropriate size. For the eyestalk of *Astacus trowbridgei,* used in this study, a trephine measuring about 2 mm. in outside diameter was satisfactory.[2] The center-pin of the trephine was placed, as shown in Figure 2, slightly posteriorad of the long axis of the stalk and approximately in the center of the non-retinal portion. Rotation of the drill would cut a circumscribed disc of the exoskeleton (Fig. 3). It was found helpful to perform the trephination with the aid of a binocular dissecting microscope, using a magnification of about 18–20 ×, and employing a source of bright illumination such as an adjustable-focus microscope lamp. Once the disc of exoskeletal material had been cut thru, it was removed after carefully separating it from the underlying hypodermis by means of a fine knife. A series of knives of various sizes and shapes was prepared by sharpening steel needles on a hone and mounting them in convenient handles. The excise skeletal disc was placed in normal saline, to be used later in closing the opening. The exposed hypodermis, readily recognized by its numerous red chromatophores, was dissected free and also stored in saline. When this has been done and the bright light focused on the operative field, the nervous portion of the eyestalk and the outline of the sinus gland can

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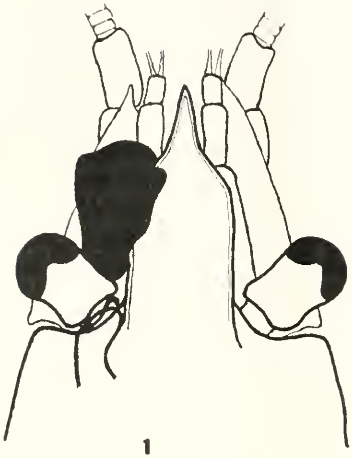
[2] Drills of assorted sizes have been made for me by Mr. H. G. Bliss, 507 Park Avenue, Herkimer, New York.



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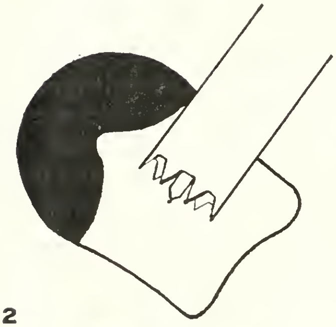

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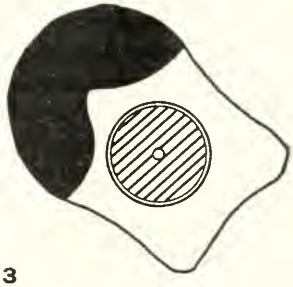

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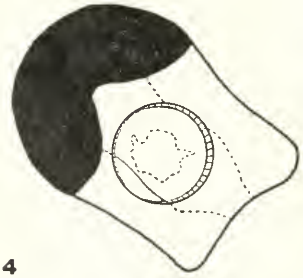

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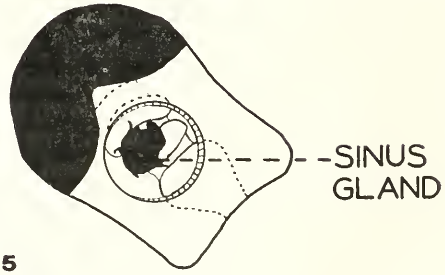

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FIGURE 1. The cephalic end of a crayfish, showing the left eyestalk prepared for removal of the sinus gland. A ligature is first loosely tied around the base of the eyestalk; this is tightened only before trephination is started. A piece of modelling clay holds the stalk in position and avoids the risk of crushing the retinal portion against the rostrum or carapace.

FIGURE 2. The left eyestalk viewed from the dorsal side, showing the location of the trephine to expose the area of the sinus gland.

FIGURE 3. The trephined area of the exoskeleton shown in diagrammatic form.

FIGURE 4. The trephined disc of skeletal material has been removed; the hypodermis, lying directly underneath the exoskeleton has also been excised. In dotted outline are shown the optic ganglia and the sinus gland, enclosed in a thin transparent sheath. The muscles of the eyestalk are not shown.

FIGURE 5. The connective tissue sheath has been removed, showing the gland and its processes located between the 2nd and 3rd optic ganglia.

be detected with the binocular microscope. Some of the muscles of the stalk lie alongside the nerve ganglia, but can be readily separated from the latter by a fine knife. The operative field at this point is diagrammed in Figure 4. A thin, transparent sheath encloses the optic ganglia and the sinus gland. This sheath is carefully slit, and the incision extended around the margin of the trephined area. In the light of the microscope lamp the sinus gland now appears as a bluish-white structure, quite easily distinguishable from the nervous portion of the stalk (Fig. 5).

The sinus gland is located between the second and third optic ganglia, in a somewhat dorso-posterior position with regard to the optic tract. In the crayfish (*Cambarus* and *Astacus*) it is not as compact a structure as is found in many of the marine crustaceans; a few filaments or processes of the gland are found to extend to adjacent ganglia; in the eyestalk of *Astacus* this seems to be less pronounced than in *Cambarus*.

After the gland has been exposed, the processes and body of the gland are carefully separated from the optic ganglia by a knife, and the entire structure removed with the aid of watchmaker's forceps and iridectomy scissors. The operative field is examined with the binocular microscope to verify complete excision; fragments of the filaments that have remained behind can be removed with the forceps.

Following removal of the sinus gland, the hypodermis, previously excised and saved in saline, is replaced to fill the gap and the trephined disc of skeletal material is fitted into the opening in the exoskeleton of the eyestalk. The groove between the margin of this opening and the rim of the disc is filled with fibrin foam. This material originally consisted of samples from the laboratory of Professor E. J. Cohn of the Harvard Medical School; later, commercial preparations, "Gelfoam, No. 12" manufactured by the Upjohn Company, proved equally satisfactory. The ligature around the base of the eyestalk is removed. The slight oozing of blood that follows in the trephined area soon leads to clotting in contact with the fibrin foam. To facilitate this clotting and the desired fixing-in-place of the skeletal disc, the animal is removed from the operating board and placed in a vessel containing water to a depth not much greater than an inch; after 2–3 hours the operated animal is replaced in its tank of deeper water, with little risk that the trephined disc will be loosened and the area of operation exposed.

In practice I have found that the operation from anesthesia to gland removal and replacement of the disc requires about 15 minutes. The initial attempts required as long as an hour, until some facility in the procedure was attained.

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THE COMPARATIVE TOLERANCES OF SOME FOULING ORGANISMS TO COPPER AND MERCURY *

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The ability of marine organisms to attach to surfaces coated with ship-bottom paints depends on the toxicity of the surface and the sensitivity of the organisms to the toxic. Pyefinch (1946) presents a table showing the sensitivity of various organisms to toxics determined primarily from analysis of their settlement on toxic surfaces at Millport, Scotland. In the present studies carried out in the Biscayne Bay area, Florida, the tolerances of several common fouling organisms to copper and mercury have been compared by observations of the sequence of attachment to antifouling paints of moderate toxicity, to series of paints of graded toxicity, and to nontoxic surfaces adjacent to antifouling paint.

RESULTS

An indication of the relative tolerance of various organisms to toxic paints can be obtained by observing the frequency of their attachments to paints which are beginning to fail after long exposure. It may be expected that the most tolerant forms will be the first to attach. The organisms attaching earliest to each of 662 antifouling paints exposed at Miami Beach and Tahiti Beach, Florida, were recorded with the following result:

Barnacles	67%
Encrusting bryozoans	18
Hydroids	9
Erect bryozoans	5
Tunicates	1
Tubeworms	< 0.1
Sponges	< 0.1

The order of attachment depends not only upon the tolerance of the organisms to the toxics, but also upon their seasonal occurrences. For example, hydroids and those forms following them in the list attach first only when barnacles and encrusting bryozoans are absent from the fouling population. Encrusting bryozoans rank below barnacles because they are absent during a large part of the year, while barnacles are present at almost all times. One of the encrusting bryozoans, *Watersipora cucullata*, appears to be somewhat more tolerant to copper than the most tolerant barnacle species, *Balanus amphitrite*, since it frequently attached earlier when both organisms were present.

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* Contribution No. 385 from the Woods Hole Oceanographic Institution. The observations described were made during an investigation of fouling by the Woods Hole Oceanographic Institution under contract with the Bureau of Ships, Navy Department, which has given permission for their publication. The opinions presented here are those of the author and do not necessarily reflect the official opinion of the Navy Department or naval service at large. The author is indebted to Dr. B. H. Ketchum and Dr. A. C. Redfield for helpful advice and criticism.

The larva of a fouling organism which is unable to attach to a toxic paint surface may attach to a more resistant form which has already become established on the surface. Following this the adult may then spread over the paint surface. The relative tolerances of some fouling organisms to the toxic surfaces can frequently be assessed from these conditions of attachment.

The barnacles, *Balanus amphitrite* and *Balanus improvisus*, and the encrusting bryozoan, *Watersipora cucullata*, were found attached directly to paint surfaces. Attaching to and growing on these forms were *Schizoporella unicornis*, *Anomia* sp., *Bugula avicularia*, *Pennaria tiarella*, *Lepas anatifera* and tunicates. Examples of several of these organisms which were unable to attach to antifouling paints, but which attached to other forms on the paint, are shown in Figures 1–5.

A more precise evaluation of tolerance is given by comparing the frequency of attachment of different organisms on a surface of moderate toxicity with their frequency on a nontoxic control surface. A paint of moderate toxicity will allow attachment only of the forms having the highest tolerance to the paint toxics. Glass panels coated on one side with a paint in which cuprous oxide served as the toxic were exposed for periods of one month. The uncoated side served as a control. The use of the glass panel gave both surfaces the same color, thus eliminating any differential effect of color on attachment. Table I shows the numbers of barnacles of three species collected on these two surfaces. The total numbers of barnacles attaching to the toxic paint surface are less than one per cent of those growing on the nontoxic control. *Balanus amphitrite* comprises over 90 per cent of the population on the toxic surface, being the only barnacle species on most panels, though it is only 7.5 per cent of the population attaching to the control surface. In contrast *Balanus improvisus* makes up 90 per cent of the total attachment to the nontoxic surface but comprises only 9.4 per cent of the population on the paint. *Balanus eburneus* is completely inhibited from the toxic surface. It is thus evident that each of these three species of the same genus demonstrates a different degree of tolerance to the copper paint.

TABLE I

Total number of barnacles attached to 33 nontoxic and toxic surfaces (80 square inches in area) exposed for one month at intervals between October 1944 and May 1946

Barnacle species	Barnacles on nontoxic surface		Barnacles on toxic surface	
	Total	% of total	Total	% of total
Balanus improvisus	17,959	89.7	15	9.4
Balanus amphitrite	1,503	7.5	145	90.6
Balanus eburneus	564	2.8	0	0.0

In addition to the barnacle fouling the only other significant attachment to this paint surface was the encrusting bryozoan *Watersipora cucullata* and the green alga *Enteromorpha* sp. Both of these species attached only during the periods of their maximum abundance. On the nontoxic panels a total of twenty species of fouling

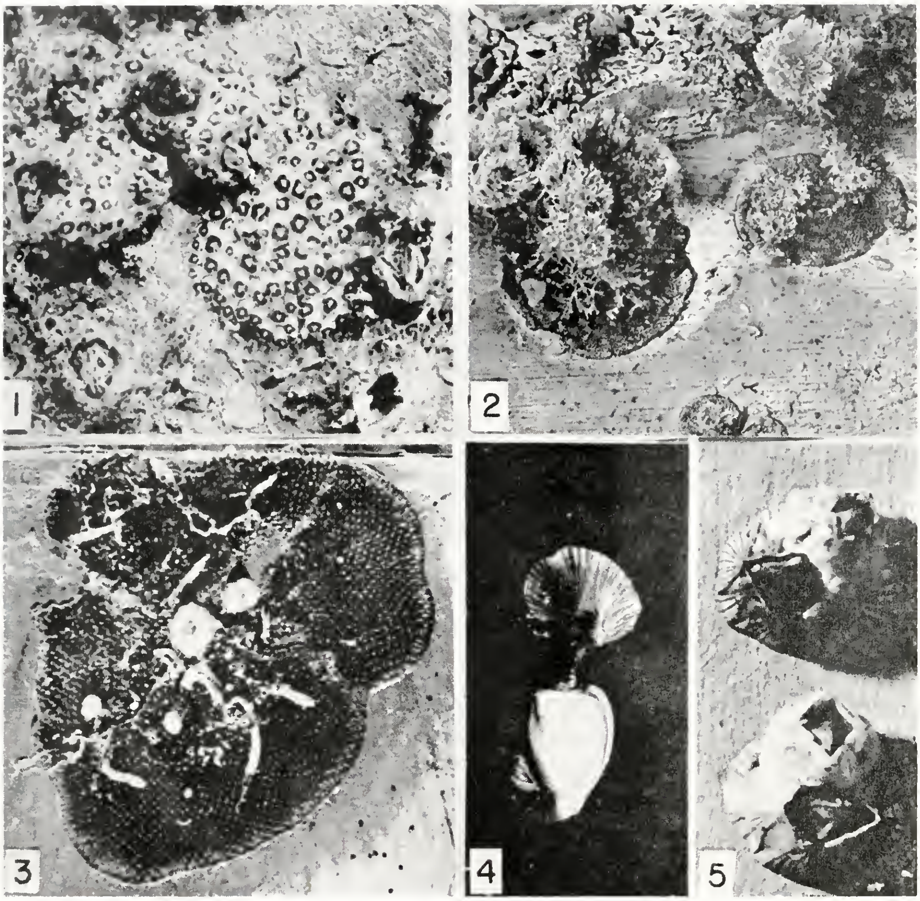

PLATE I

FIGURE 1. A speckled tunicate limited in attachment to barnacles and *Anomia* sp. without spreading onto the paint surface.

FIGURE 2. *Bugula avicularia* attached to the encrusting bryozoan, *Watersipora cucullata*, which is growing directly on the paint.

FIGURE 3. Barnacles attached to *Watersipora cucullata* which developed directly on the paint surface. Tubes of *Dasychone conspersa* are also attached on *W. cucullata*.

FIGURE 4. Gooseneck barnacle, *Lepas anatifera*, attached to *Balanus amphitrite* which attached to an otherwise effective antifouling paint on a ship's bottom.

FIGURE 5. Two examples of *Balanus improvisus* which attached to the more resistant barnacle, *B. amphitrite*. Photographed after removal from the paint surface.

organisms attached during the period covered by these observations. These must be considered to be less tolerant than the four organisms which attached to the paint surface.

In order to establish the relative tolerances to copper of the more sensitive fouling forms a set of paints containing graded amounts of copper flake pigment was prepared. The copper leaching rates of these paints were measured as described by

Ketchum, Ferry, Redfield, and Burns (1945) and were found to vary in proportion to the pigment content and to decrease with the time of exposure. This series thus provided graded toxicities.

The relative tolerance of attaching organisms could be judged by the time of exposure elapsing before they attached to any one member of the series or by the number of members of the series which were fouled at any one time. The paints were applied to 8 × 10-inch weldwood panels which were immersed at Miami Beach and Tahiti Beach, Florida. The composition of the paints and the time of exposure before various fouling forms attached are given in Table 11.

Table 11

Number of months of exposure prior to appearance of fouling on painted panels immersed in the sea at Miami Beach, Florida (May 2, 1945–March 2, 1946), and at Tahiti Beach, Florida (May 2, 1945–September 2, 1945). The paints contained graded amounts of copper flake in a vehicle consisting of equal parts, by weight, of polyvinyl butyral and rosin.

Paint number Copper content, per cent dry weight	AF12 90	121 67	122 45	123 30	124 23	125 14
Miami Beach						
Balanus amphitrite	4	3	3	2	1	1
Watersipora cucullata	9	9	8	2	2	1
Balanus improvisus	a	a	a	a	2	1
Tahiti Beach						
Polysiphonia sp.	3	2	2	2	2	2
Balanus amphitrite	b	b	4	4	3	1
Hydroides parvus	b	b	b	4	4	3

a. None of this species had attached to these paints after 10 months of exposure.
b. None of these species had attached to these paints after 4 months of exposure.

At Miami Beach only three fouling organisms attached to the copper paints, two species of barnacles and the encrusting bryozoan *Watersipora cucullata*. Throughout the period of exposure there was no marked seasonal variation in the incidence of barnacles. However, seasonal fluctuation in the incidence of *Watersipora cucullata* accounts principally for the nonattachment of this organism between the second and ninth months of immersion of the paints. Its absence from the paints is, therefore, not indicative of its sensitivity to the paint toxic.

The exposure at Tahiti Beach, Florida, indicated that, on these copper paints, a red alga of the genus *Polysiphonia* is more tolerant, and that the tube worm, *Hydroides parvus*, is less tolerant than *Balanus amphitrite*.

Some species of fouling were too sensitive to attach even to the paints of lowest toxicity. To ascertain their relative tolerance of paint toxics their sequence of attachment was noted on nontoxic areas adjacent to paint surfaces. Observations by Miller (1946) on the diffusion of toxics from antifouling paints indicate that toxic gradients exist, decreasing from the paint edge. To provide nontoxic areas for attachment adjacent to the paint surfaces, strips 5, 10, and 20 mm. in width were left free of paint on the panels of the graded toxicity series.

At both exposure locations colonial tunicates attached to the widest bare strips on the paints of lowest toxicity, AF124 and AF125, by the second month of immersion.

By the third and fourth months of exposure hydroids had attached to the initial fouling on these nontoxic areas. These observations indicate that tunicates are more tolerant than hydroids to copper but less tolerant than those forms able to attach directly to the paint surface.

From the preceding observations several fouling forms can be arranged in order of decreasing tolerance to copper as follows: *Polysiphonia* sp., *Watersipora cucullata, Balanus amphitrite, Balanus improvisus, Balanus eburneus, Hydroides parvus,* tunicates and hydroids.

In the examination of antifouling paints employing mercury pigments or mixtures of mercury and copper pigments as the toxic ingredients, it was noted that certain of the fouling organisms appeared to be more sensitive to mercury than to copper while for others the reverse was true.

To establish the comparative tolerance of different species to mercury a series of paints containing mercurous chloride in graded concentration was made. The vehicle was the same as that used for the copper series. The mercury content of these paints was equal in weight to the corresponding member of the copper series. These paints were immersed simultaneously and at the same locations as the copper paints. The extent of exposure before fouling attached to the paint surfaces of the AF12 mercury series is shown in Table III.

TABLE III

Number of months of exposure prior to appearance of fouling on painted panels immersed in the sea at Miami Beach, Florida (May 2, 1945–March 2, 1946), and at Tahiti Beach, Florida (May 2, 1945–September 2, 1945). The paints contained graded amounts of mercury, added as mercurous chloride, in the vehicle described in Table II.

Paint number Mercury content, per cent dry weight	AF12 90	121 67	122 45	123 30	124 23	125 14
Miami Beach						
Balanus amphitrite	9	9	2	2	2	1
Balanus improvisus	a	a	a	4	9	2
Bugula neritina	a	a	a	2	6	1
Watersipora cucullata	a	a	a	6*	a	4*
Tahiti Beach						
Polysiphonia sp.	b	b	b	3	3	1
Balanus amphitrite	b	b	b	b	2	2

a. None of these species had attached to these paints after 10 months of exposure.
b. None of these species had attached to these paints after 4 months of exposure.
* Single specimen not present on following month.

At Miami Beach four organisms attached to this series of paints. The most resistant of these was *Balanus amphitrite*, followed in order by *B. improvisus* and *Bugula neritina*. *Watersipora cucullata*, which was found to be quite tolerant of copper, attached to only two of these paints, and in neither case was the attachment secure. In contrast, *Bulgula neritina* was able to attach directly to the mercury paints but was never found on any of the comparable copper paint surfaces. At Tahiti Beach *Polysiphonia* sp. was found to be more tolerant than *Balanus amphitrite* to the mercury paints.

Photographs of two of the copper and two of the mercury paints of these series, after nine months of exposure at Miami Beach, are shown in Figure 6. The ability of *Watersipora cucullata* to attach to the copper paints and its absence from the comparable mercury paints are shown. Both of the copper paints and one of the mercury paints are fouled with *Balanus amphitrite*.

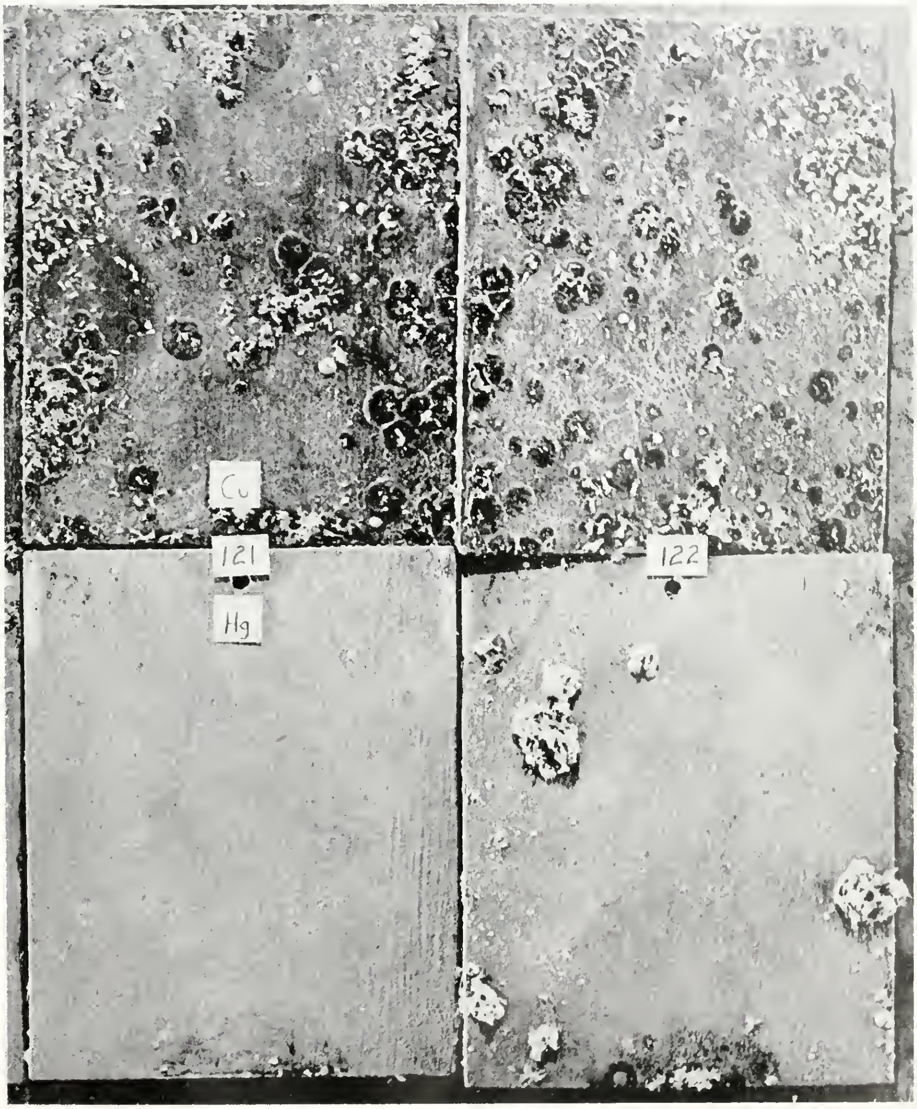

PLATE II

FIGURE 6. Comparison of the attachment of *Watersipora cucullata* to paints pigmented with copper and mercury after nine months of exposure. *W. cucullata* appears on the copper series (top panels) as the dark disc-like organisms.

Nontoxic strips on panels otherwise coated with the mercury paints permitted attachment of the more sensitive fouling forms. The more resistant forms mentioned above always attached first to these nontoxic areas. In addition, *Watersipora cucullata*, *Anomia* sp., hydroids and tunicates invaded the unpainted areas at Miami Beach. At Tahiti Beach *Enteromorpha* sp. and *Hydroides parvus* attached in the order given.

These exposures indicate the following order of decreasing tolerance of some fouling organisms to mercury as a paint toxic: *Polysiphonia* sp., *Balanus amphitrite*, *Bugula neritina*, *Balanus improvisus*, *Watersipora cucullata*, *Anomia* sp., *Enteromorpha* sp., *Hydroides parvus*, hydroids and tunicates.

From the results of the various methods described above the comparative tolerance of several fouling organisms to copper and mercury has been evaluated. The results are given in Table IV. The organisms listed as attaching to adjacent nontoxic surfaces include those which may on occasion be found on paints of low toxicity but which generally do not attach directly to the paint surface. The exact position in the order of decreasing tolerance, for all but the first three organisms in each column, requires further verification.

TABLE IV

The comparative tolerance of some fouling organisms of the Biscayne Bay area, Florida, to copper and mercury employed as pigments in antifouling paints. The lists are arranged in approximate order of decreasing tolerance.

	Copper	Mercury
Organisms found attached to toxic paint surfaces	*Polysiphonia* sp. *Watersipora cucullata* *Balanus amphitrite* *Enteromorpha* sp. *Balanus improvisus* *Balanus eburneus* *Hydroides parvus*	*Polysiphonia* sp. *Balanus amphitrite* *Bugula neritina* *Balanus improvisus* *Watersipora cucullata*
Organisms found mainly attached to nontoxic areas adjacent to paint surfaces	*Anomia* sp. *Bugula neritina* *Bugula avicularia* *Lepas anatifera* tunicates hydroids	*Anomia* sp. *Enteromorpha* sp. *Hydroides parvus* hydroids tunicates

DISCUSSION

Relatively few of the sessile species in the Miami area which attach to neutral surfaces are able to attach to antifouling paints. Even paints of such low toxicity that they become completely covered permit the attachment of only four or five forms. Many less resistant fouling organisms are excluded from the paint surface. Effective antifouling paints must be designed to prevent the attachment of the few most tolerant forms.

The species most tolerant of copper are different from those most resistant to mercury. The conflicting opinions which have existed concerning the relative efficiencies of copper and mercury compounds as paint toxics may depend largely on the species of fouling organisms present at the particular testing location.

The present results and those given by Pyefinch (1946) agree in showing that *Enteromorpha* and *Polysiphonia* are among the more resistant forms, and *Anomia* and tunicates have low tolerance to paint toxics. Tubularian hydroids were noted only as secondary fouling at Miami, though Pyefinch rates them more resistant than *Balanus* and equal to *Enteromorpha*. A direct comparison of the results with *Balanus* is not feasible since the three species found in Miami showed marked differences in toxic sensitivity. None of these species are found at Millport where Pyefinch's studies were made. Pyefinch lists a greater variety of algae, including diatoms, which were not included in the investigations at Miami.

SUMMARY

The comparative toxic tolerance of several fouling organisms was based on the sequence of their attachment to copper and mercury antifouling paints. A red alga, *Polysiphonia* sp., and the barnacle, *Balanus amphitrite,* were the most tolerant and attached to copper and mercury paints before other organisms. The encrusting bryozoan, *Watersipora cucullata,* was found to be slightly more tolerant to copper but considerably less tolerant to mercury than the above two forms. Less tolerant forms were *Balanus improvisus, Hydroides parvus, Bugula neritina, Anomia* sp., *Enteromorpha* sp., tunicates and hydroids. Other species, though attaching to nontoxic surfaces, were never found on the toxic paints.

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SYSTEMATIC SEROLOGY AMONG CERTAIN INSECT SPECIES

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Introduction

The measure of the biochemical similarity among organisms is of interest to all persons doing research whose ultimate goal is to determine the essential nature of organisms. A natural consequence of studies in this direction is the systematic classification of organisms based on the similarities and differences in their serological behavior. The blood sera and the soluble protein constituents have been the chief substrates through which serological classifications have been made. Extractions of whole organisms such as insects have also proved to be adequate for comparing the similarity among insect species (Leone, 1947). Systematic serology does not necessarily answer questions on the phylogenetic origin of organisms, but rather associates animals as they exist today into natural classifications based on their essential biochemical similarities.

The following paper is designed to show the type of serological results that can be expected when various families in the insect order Orthoptera are examined serologically. The organisms used in these tests are listed below:

Family	Scientific name	Common name
Acrididae	*Melanoplus femur-rubrum* DeG.	Red-legged grasshopper
	Melanoplus differentialis Thos.	Differential grasshopper
	Romalea microptera Beauv.	Florida lubber grasshopper
	Paroxya atlantica Scudd.	Atlantic locust
	Leptysma marginicollis Serv.	Slender locust
	Spharagemon bolli Scudd.	Boll's locust
	Arphia xanthoptera Burm.	Yellow-winged locust
Tettigoniidae	*Conocephalus strictus* Scudd.	Straight-lanced grasshopper
	Conocephalus fasciatus DeG.	Slender-meadow grasshopper
Mantidae	*Paratenodera sinensis* Sauss.	Chinese mantis
Gryllidae	*Gryllus assimilis* Fabr.	Field cricket
Blattidae	*Periplaneta americana* (Linn.)	American cockroach

Materials and Methods

Preparation of antigens

After first removing the tarsi and the wings the insects were ground in a mortar with sand. Sufficient buffered 0.85 per cent NaCl was added to prevent drying and denaturation of the saline soluble components. The resulting emulsions were cleared using the same techniques described in a previous paper by the author (Leone, 1947). Micro-Kjeldahl nitrogen determinations were performed to determine the total nitrogen and the non-protein nitrogen. Values for the protein content of the antigens were determined as follows: grams total nitrogen $-$ grams non-protein nitrogen $=$ grams protein nitrogen $\times 6.25 \times 100 =$ grams per cent protein in solution. These values are given in Table I. When only a few insects of a given species were available, it was necessary to dilute the extract sufficiently to obtain volumes large enough to conduct the projected tests. As a result of this the concentrations of protein in solution were markedly reduced in some antigen extracts.

TABLE I

Protein concentrations in extracts of Orthoptera

Organism	Key	Grams protein per cent
Melanoplus femur-rubrum	GR	0.32
Melanoplus differentialis	GD	0.22
Romalea microptera	GF	0.07
Paroxya atlantica	GA	0.05
Leptysma marginicollis	GS	0.03
Spharagemon bolli	GB	0.10
Arphia xanthoptera	GY	0.06
Conocephalus strictus	GL	0.04
Conocephalus fasciatus	GM	0.05
Paratenodera sinensis	MC	0.09
Gryllus assimilis	KC	0.13
Periplaneta americana	CA	0.82

Preparations of antisera

A series of four doubling doses of antigen were injected intravenously in rabbits on alternate days; initial injection was one ml. A second series of antigen injections were given to a rabbit if a preliminary bleeding and testing seven days after the last injection revealed little or no response to the antigen. Final bleedings by cardiac puncture were performed on the eighth day after the last injection. The blood was permitted to clot for 24 hours. The expressed sera were centrifuged, sterile filtered through a Seitz filter, bottled in serum vials, and stored in the refrigerator.

Methods of testing

Ring tests were performed in accordance with standardized procedures as outlined by Boyden (1926).

Photoelectric measurements of precipitin turbidities were made using the Libby photronreflectometer (photron'er) (1938). Titrations were performed and results plotted using the technique of Boyden and DeFalco (1943).

EXPERIMENTAL RESULTS

The production of immune sera against all the Orthoptera except the Blattidae (roaches) proved to be difficult. Protein concentrations were low, and this fact, together with the small quantities of material available restricted the number of injections that could be used to produce an antiserum, and also restricted the number of tests that could be carried out with any one antigen. The photron'er curves proved to have low peaks and broad bases. Inter-family relationships could be established with these curves. Ring test reactions, in general, confirmed the whole curve comparisons of the insect antigens, in so far as this limited technique can be compared with the nephelometric photron'er method. Typical sets of data are presented to show the correspondence obtained by the two methods (Figs. 1 and 2). A summary of all the information obtained using both techniques is presented in Table II.

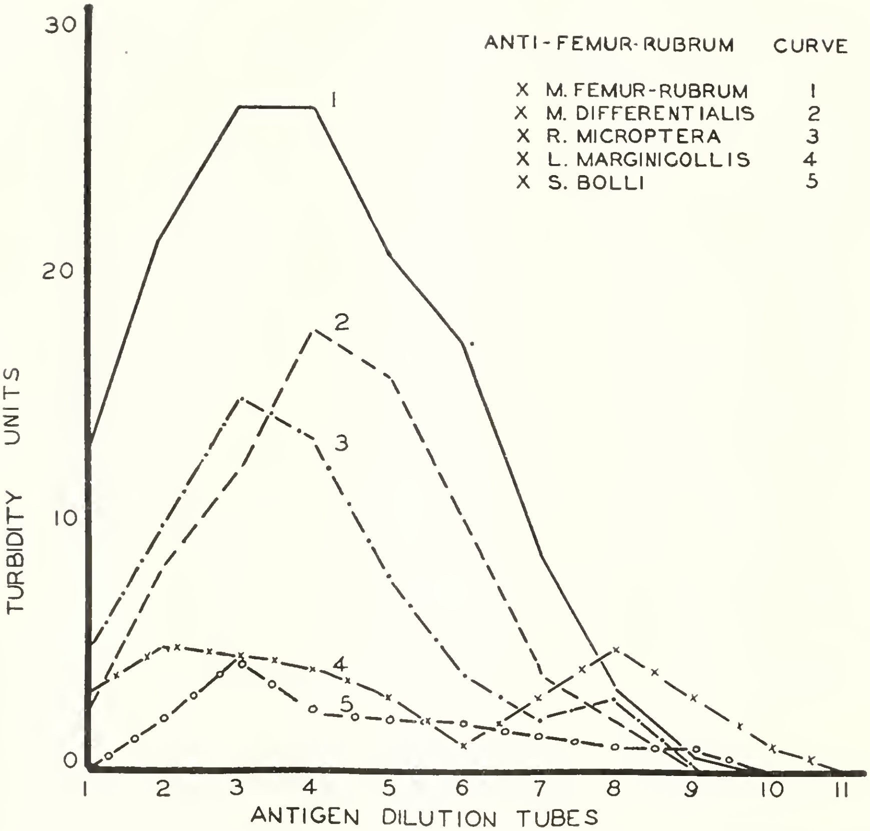


FIGURE 1. A photron'er curve series titrated against an anti-*Melanoplus femur-rubrum* serum. Percentage values indicate the relative degrees of correspondence to the homologous antigen *Melanoplus femur-rubrum*, demonstrated by the various heterologous antigens. The undiluted antiserum shows a low order of turbidities but a broad reactivity range.

Family Acrididae (Short-horned grasshoppers)

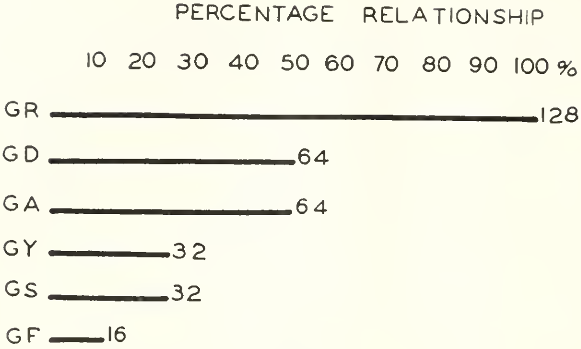
Antisera sufficiently powerful to be recorded on the photron'er were secured in only two instances, one against the Florida Lubber grasshopper (*Romalea microptera*), and the other against the Red-legged grasshopper (*Melanoplus femur-rubrum*). The latter antiserum was obtained as the result of a double series of injections separated by a period of eight days between the first and second injection series.

The anti-Lubber grasshopper serum showed only a slight discrimination between the Red-legged and the Differential grasshoppers (*Melanoplus differentialis*).

TABLE II

Comparison of ring test and photron'er test results

	Antisera	Homologous titer in thousands	Antigens (Relationship values in per cent)											
			Red-legged grasshopper	Differential grasshopper	Florida lubber grasshopper	Atlantic locust	Boll's locust	Yellow-winged locust	Slender locust	Slender-meadow grasshopper	Straight-lanced grasshopper	Chinese mantis	Field cricket	American roach
Photron'er Tests	Red-legged grasshopper		100.0	54.9	50.7	44.7		29.2	23.8		12.4	0.0	0.0	0.0
	Florida lubber grasshopper		13.5	9.6	100.0							0.0	0.0	0.0
	Chinese mantis		0.0	0.0	0.0	0.0	0.0	0.0		0.0	0.0	100.0	6.2	18.7
	Field cricket		6.0	4.5			7.3		2.8			10.5	100.0	29.0
	American roach		0.0	0.0				0.0		0.0	0.0	0.0	4.3	100.0
Ring Tests	Red-legged grasshopper	256	100.0	50.0	25.0	50.0		50.0	50.0		12.5	6.8	12.5	6.8
	Differential grasshopper	32	100.0	100.0		50.0								
	Florida lubber grasshopper	64	12.5	3.4	100.0	0.0	0.0	0.0		0.0		0.0	0.0	0.0
	Atlantic locust	32	100.0			100.0	0.0	0.0	0.0			0.0	0.0	0.0
	Chinese mantis	256	0.0	0.0		0.0	0.0	0.0	0.0		25.0	100.0	25.0	25.0
	Field cricket	256	6.8		6.8	0.0	0.0	0.0	0.0		12.5	6.8	100.0	25.0
	American roach	256	6.8	6.8		0.0	0.0	0.0	0.0		12.5	6.8	25.0	100.0
	Red-legged grasshopper (Diluted 1+1)	128	100.0	50.0	12.5	50.0		25.0	25.0		0.0	0.0	0.0	0.0



Key	*Antigens*
GR	Red-legged grasshopper
GD	Differential grasshopper
GA	Atlantic locust
GY	Yellow-winged locust
GS	Slender locust
GL	Straight-lanced grasshopper
GF	Florida lubber grasshopper

FIGURE 2. A typical plot of ring test titers obtained by heterologous antigens titrated against an anti-*Melanoplus femur-rubrum* serum. The number behind each line represents the highest dilution, in thousands, at which a definite interfacial ring was obtained. Ring tests performed using undiluted antiserum gave undifferentiating titers among all the *Acrididae* except the Lubber grasshopper. A dilution of the antiserum with equal parts of saline gave the above discrimination.

The anti-Red-legged grasshopper serum demonstrated an ability to react against many heterologous antigens. Even closely related forms could be readily distinguished, however of the species that showed reactivity, the Straight-lanced grasshopper (*Conocephalus strictus*) belonging to the more distant Tettigoniidae, showed the least amount of turbidity. The mantids, crickets, and roaches produced no heterologous turbidities in the photron'er and thus indicated their more distant relationship.

The confirmation of the reduced reactivity of these more distant species when tested with the ring test was striking. Intra-family differences could not be demonstrated with the ring test (exception may be cited in the case of the Lubber grasshopper *Romalea microptera*). A dilution of one part serum to one part saline reduced the reactivity of the antiserum to such an extent (and also increased its specificity) that all organisms outside the family no longer reacted.

Antisera procured against other members of the family Acrididae, i.e., the Differential grasshopper and the Atlantic locust were too weak to give photron'er readings. Ring test titers for these organisms were very low also, the homologous reactions having a titer of only 1 to 32000.

Family Mantidae (Praying mantids)

Only a single species of this family, the chinese mantis (*Paratenodera sinensis*) was secured. The photron'er results indicate a very specific antiserum with the families Blattidae and Gryllidae barely making their appearance. The Blattidae (*Periplaneta americana*) show a closer relationship to the Mantidae than any other family considered.

Ring test results indicate undifferentiating heterologous titers among the cricket, roach, and Straight-lanced grasshopper, representing their respective families Gryllidae, Blattidae, and Tettigoniidae when compared against the Mantid. The family Acrididae, evidently more distant, did not react.

Family Gryllidae (Crickets)

In view of the morphological indistinctness of the several large black species of crickets, all the specimens collected were pooled and treated as a single type species (*Gryllus assimilis*) to represent this family. By photron'er test the *Blattidae* (roaches) showed the closest relationship; other families, on the whole, reacted weakly. Greater differentiation of these distant relatives was shown by the ring tests.

Family Tettigoniidae (Long-horned grasshoppers and katydids)

Two species were secured to represent this family, the Straight-lanced grasshopper (*Conocephalus strictus*) and the Slender-meadow grasshopper (*Conocephalus fasciatus*). Antisera were not obtained against these antigens. Both species were used however in the heterologous reactions to aid in establishing their position with respect to other species and the position of other species with respect to them.

Family Blattidae (Roaches)

One sample of the American cockroach (*Periplaneta americana*) was chosen to represent this family. The antiserum was the most powerful of all those tested, i.e., gave the greatest turbidity readings, but also was among the most specific of the antisera with respect to inter-family reactions on the photron'er. The field encompassed by means of the ring test technique is somewhat broader than the photron'er examination of this antiserum. The Gryllidae reactions were the most nearly like the Blattidae, of all the families tested. The Tettigoniidae, Acrididae, and the Mantidae showed lessened but approximately the same degrees of relationship to the Blattidae.

Discussion

The results as presented above are at best only a beginning in the study of the problem of the quantitative systematic serology of insects. The insect species used in the tests were chosen because of their availability to the writer. No attempt was made to choose species in such a way, or to perform tests in such a manner as to solve particularly significant problems in insect relationships. It was desired to know the type of serological results that could be obtained using extracts of whole insects as antigens characteristic for the species tested.

The results do indicate the feasibility of making a quantitative serologic analysis of representatives of the families, genera, and species of insects.

Boyden (1943) has been able to establish relatively constant inter-species, intergeneric, and inter-family relationships among the Crustacea. His average value for the relationship of sera of the same genus is 46 per cent; the averaged value for relationships of genera in the same family is 30 per cent; and, for inter-family relationships the average is 9 per cent.

Intra-generic values for the two species of Melanoplus considered in this paper have a value of 54.7 per cent. The averaged relationships of extracts of genera within the insect family Acrididae proved to have a value of 28 per cent. Inter-family relationships for the order Orthoptera have an average value of 11.5 per cent. The extremes of the serological relationship values for any given group using extracts of fresh insects are all within the extreme values reported by Boyden, who used whole sera for his comparisons. The averaged values obtained for relationships between species, genera, and families are in accord with the Crustacea values.

More information will have to be acquired on the nature of serological reactions, and more data accumulated on actual systematic tests before serological limits in terms of percentages, or other statistical rankings, can be summarized or defined as representing "species," or "genera," or "families" of insects.

Tentative serological relationships for four of the five families of Orthoptera investigated are given in Figure 3. The fifth family tested, the Tettigoniidae are not represented in Figure 3 inasmuch as no antiserum was produced against this family and reciprocal relationships could not be established. It should be pointed out that the values given are not absolute but only relative. A summary of the relationships of these Orthoptera as determined by the comparison of extracts made from whole insects is as follows:

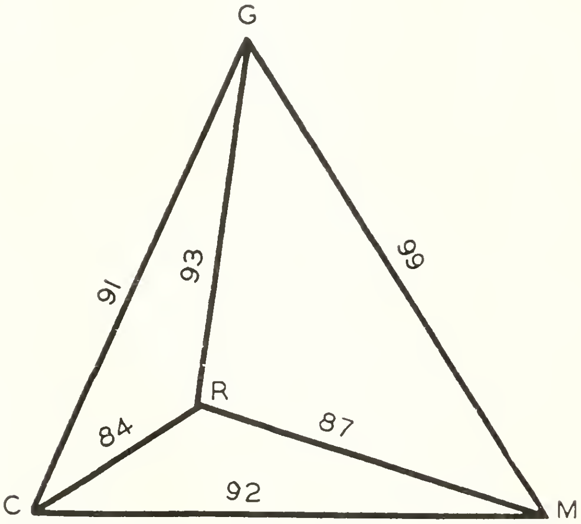


FIGURE 3. A diagram to show the relative distances of four families of Orthoptera from each other. The data are tentative inasmuch as the families have not yet had really adequate testing. The families concerned are Blattidae (*R*), Mantidae (*M*), Gryllidae (*C*), and Acrididae (*G*). The figure requires three dimensions for adequate representation, hence values contiguous to the Blattidae locus are not in proportion with the remainder of the figure. A value of 99 represents the maximum measurable distance between any two families.

A. Blattidae—approximately equidistant from the Mantidae and Gryllidae and closer to these families than to the Acrididae.

B. Mantidae—most closely related to the Blattidae. Approximately equally related to the more distant Gryllidae and Acrididae.

C. Gryllidae—closer to Blattidae than to Mantidae and Acrididae.

D. Acrididae—appears to be more closely related to Gryllidae and Blattidae, than to the Mantidae.

E. Tettigoniidae—most distant from the Mantidae and Blattidae of all the families tested. Shows slightly more relationship to the Acrididae than to the other families.

There is striking correlation between the findings of Crampton (1932) in his taxonomic and phylogenetic studies and the orientation of insect families as discussed above, indicating that there may be some correlation between time of origin and degree of similarity in the antigenic constituents of insects, i.e., their serum proteins and other extractable proteins. Except for the position assigned to the Gryllidae, there is also general agreement between the phylogenetic tree of Walker (1922) and the relative positions occupied by the five families of Orthoptera investigated in this paper. As stated previously, however, systematic serology does not attempt to give a final answer to questions on the phylogenetic origin of organisms, but rather associates animals as they exist today into natural classifications based on their essential biochemical similarities. Where serological evidence is in agreement with a paleologic-taxonomic study which correlates phylogenetic origin and present day classifications, then the biochemical (i.e., serological) evidence becomes additive to the other two and increases the likelihood that such a study presents the true picture of the evolutionary development of the species examined.

SUMMARY

1. Representatives of five families of the insect order Orthoptera were compared serologically.

2. On the basis of the degree of serological similarity among them the relative positions of these five families are given.

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THE ACTION OF ELECTRICAL STIMULATION AND OF CERTAIN DRUGS ON CARDIAC NERVES OF THE CRAB, CANCER IRRORATUS

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INTRODUCTION

The nervous regulation of the decapod crustacean heart has been studied for over a century, yet the nature of the mechanisms involved are still obscure. The nerves concerned are minute, and only in recent years has a picture been obtained of the anatomical relationships of the cardio-regulatory nerves to the heart and its ganglion cells.

Inhibitory cardiac nerves in crayfish were early indicated or demonstrated by several investigators including Dogiel (1876, 1877), Yung (1878), and Plateau (1878, 1880), whose work showed in a general way that these nerves arise from the anterior part of the thoracic ganglionic chain or mass. Dogiel believed that nerves arising anterior to the sternal artery and running to the pericardium and abdominal extensor muscles were inhibitory, but as his only points of electrical stimulation were the pericardium and the thoracic nerve cord between the bases of the second and third leg nerves it does not appear that he traced the inhibitory nerves in detail. In view of frequent misquotations it is well to note that "Dogiel's nerve" was never claimed to run along the sternal artery or actually to enter the heart. Inhibitory cardiac nerves in the crayfish have recently been traced by Wiersma and Novitski (1942).

Definitive information on the course of inhibitory cardiac nerves in crabs rests upon the work of Jolyet and Viallanes (1892, 1893), Conant and Clark (1896), and Bottazzi (1901). Conant and Clark demonstrated most clearly in *Callinectes* that the inhibitory nerves arise as a single pair close to the bases of the recurrent cutaneous nerves, and run with these large nerves anterodorsally from the thoracic ganglion, eventually separating and joining the cardio-accelerator nerves on each side to form the lateral pericardial plexus. The entrance of the inhibitory nerves into the heart was not observed by Conant and Clark, but the observations of Alexandrowicz (1932), Heath (1941), and the writer are in agreement that the cardio-regulatory nerves enter the crab heart as a single dorsolateral pair of delicate strands, containing very few fibers.

Interest in cardio-accelerator nerves at first centered about "Lemoine's nerve," which was described in the crayfish by Lemoine (1868) as arising from the stomatogastric nervous system on the anterior dorsal wall of the stomach. This nerve, which is extremely fine, was reported to run beneath the ophthalmic artery and to break up as it entered the heart. In view of later misquotations, it is well to note that Lemoine did not derive his cardiac nerve from the cerebral ganglia, nor did he produce any effects on the heart by stimulation of the "brain." The positive results of stimulation of "Lemoine's nerve" reported by several authors (Lemoine, 1868; Yung, 1878; Plateau, 1880) were possibly a reflex phenomenon. More recent workers, including Jolyet and Viallanes (1892, 1893), Conant and Clark (1896), Wiersma and Novitski (1942), have not found "Lemoine's nerve" to be a cardio-accelerator. Vital staining studies by Alexandrowicz (1932), Heath (1941) and the writer show that this nerve in the crab innervates the anterior valves of the heart, and does not pass to heart ganglion or muscle. Jolyet and Viallanes (1892, 1893), confirmed by Conant and Clark (1896), showed that the cardio-acceleratory nerves in crabs consist of two pairs arising dorsal to the bases of the nerves to the third maxilliped and the first leg. The two acceleratory nerves on each side join the single inhibitory nerve to form the lateral pericardial plexus, from which the acceleratory nerves pass into the heart in the same thin strand which carries the inhibitors.

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The cardiac ganglion (intrinsic ganglion) of the crab heart, as in other marine decapods, contains about nine cells and sends its fibers in a complex but regular pattern throughout the heart (Alexandrowicz, 1932). It is innervated by the single pair of nerves carrying inhibitory and excitatory fibers. The exact relationship of each type of fiber to the heart ganglion and heart muscle is not fully understood. Alexandrowicz and others have observed that the fibers entering the heart are of two sizes, the larger of which make synaptic contact with processes of the ganglion cells, while the smaller fibers pass to the muscle. Alexandrowicz suggests that the thicker fibers are inhibitory, the thinner excitatory. However, in their studies of the multiple innervation of crustacean limb muscles, van Harreveld and Wiersma (1939) have found that the inhibitory fiber is thinner than the motor fibers to the same muscle, while Wiersma and Novitski (1942) have shown that the cardio-acceleratory nerves in the crayfish probably act upon the heart ganglion rather than on muscle.

Pharmacological studies have indicated that a cholinergic nervous mechanism is concerned in the initiation of heartbeat in higher crustaceans (Welsh, 1939a, 1939b; Davenport, Loomis, and Opler, 1940; Davenport, 1941, 1942; Prosser, 1942 and others). The isolated decapod heart is stimulated by acetylcholine, an effect augmented by eserine and blocked by atropine. While it is generally agreed that the intrinsic ganglion has a pacemaker function, and that it is stimulated by acetylcholine (Welsh, 1942), attempts to determine whether acetylcholine acts in a muscarine-like or in a nicotine-like fashion on the ganglion or muscle of the isolated heart (Davenport, 1941, 1942) have revealed little concerning the manner of action of the regulatory nerves which reach the heart from the central nervous system.

In pharmacological studies of the isolated heart it has generally not been possible to tell at what sites the drugs used are actually taking effect. For instance, the rate of the isolated crustacean heart is quickened by acetylcholine. This effect is probably due to the action of the drug on the heart ganglion, but the possibility of a direct action on the muscle remains. The sensitivity of the ganglion to acetylcholine does not prove that the neurons of the ganglion are themselves cholinergic. They might be cholinergic, adrenergic, or of some still unknown nature. It is helpful to recall that in vertebrates cholinergic preganglionic fibers may stimulate either cholinergic or adrenergic postganglionic neurons in the autonomic nervous system. Thus the situation in the isolated crustacean heart is more complex than it may appear at first glance. When a drug is applied to the whole heart, it undoubtedly has some effect on muscle as well as on the ganglion, and if we admit the possibility of its stimulating the remnants of regulatory nerve still within the heart, we see further complications, not only because we may not be able to identify the effects of the regulatory nerves in the total response, but also because the simultaneous stimulation of these opposing nerves could produce conflicting effects. Since the completion of the work reported here, Wiersma and Novitski (1942) have shown that perfused acetylcholine and excitatory nerve stimulation produce similar effects in the crayfish heart, and that eserine augments the effect of nerve stimulation upon heart rate. These observations may be considered as excellent evidence for the cholingeric nature of the cardio-accelerator nerves and for the termination of these nerves upon the ganglion cells rather than on muscle. However, it is still not known at what point in the heart the inhibitory nerves act. Previous studies by the writer on nervous inhibition of the heart of *Panulirus* (Smith, 1940–41) led to the conclusion that the cardiac ganglion was possibly cholinergic, but that the inhibitory mechanism was not of the "muscarine-like" cholinergic type.[2]

In view of the scarcity of direct evidence on the nature of the regulatory heart nerves it was felt that further study of the effects of certain drugs on the action of these nerves in a semi-intact preparation might yield information regarding their nature and mode of action. It was originally hoped that this work could include an electrical study of the heart ganglion when the heart was being inhibited or excited by nervous action, in order to clarify the role of the heart ganglion in nervous control, but, unfortunately, exigencies of 1942 did not permit electrical studies to be made.

Throughout this work I received constant encouragement and much helpful criticism from Dr. John H. Welsh, to whom I wish to express my gratitude.

Material and Methods

The eastern rock crab, *Cancer irroratus,* was found to be of suitable size and hardiness for winter laboratory use if maintained under cold conditions. By suitable cannulation, the heart was perfused *in situ* with physiological solutions or drugs. Heart action was recorded kymographically, while stimulation was applied to the inhibitory and to the excitatory nerves by means of two sets of mechanically manipulated electrodes which were left in place upon the nerves through the course of an experiment. Exposure of the thoracic nerve mass from beneath gave access to these nerves close to their point of origin, at a considerable distance from the heart. Stimulation was supplied either by a pair of Harvard inductoria, or by a thyratron stimulator (modified after Delaunois, 1939) giving repetitive shocks at controllable frequencies and voltages.

Perfusion fluid was made up following Cole's (1940) analysis of the blood of *Cancer borealis*. (An analysis of the blood of *C. irroratus,* generously carried out by Dr. Cole, showed that the composition of the blood of this species is similar to to that of *C. borealis.*)

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[2] The statement in the original report (Smith, 1940–41) that, "the inhibitory mechanism is not of the cholinergic type," was not justified by experimental results, and should be in the less sweeping form, "not of the muscarine-like cholinergic type."

NaCl	0.506 M	1000 parts			
KCl	0.506 M	26.1 parts	$MgCl_2$	0.506 M	8.7 parts
$CaCl_2$	0.506 M	26.1 parts	$MgSO_4$	0.506 M	39.1 parts

To each liter of solution was added 17.6 cc. of 0.5 M boric acid and 0.96 cc. of 0.5 M NaOH as a buffer.

The crab heart perfused *in situ* exhibits a steady beat which may be slow or rapid, depending upon the temperature and the conditions of the dissection and perfusion. For best results, all the supporting ligaments of the heart should be intact, although a fairly good beat may often be obtained when the posterodorsal ligament has been severed. A low temperature (10° – 15° C.) is much more favorable than room temperature at any season. The pressure within the heart, which depends upon the rate of perfusion as well as upon the integrity of the heart and its valves, largely controls the amplitude and rate of beat. In diastole the heart is stretched horizontally by its elastic supporting ligaments until it presents a broad, flat or slightly depressed dorsal surface. In systole, the internal pressure causes the dorsal surface to bulge upwards as the heart passes from a flat to a more rounded cross-section, while at the same time, the rear wall of the heart bulges posteriorly. This latter motion was recorded in the tracings as a sharp upward deflection of the writing lever.

With insufficient internal pressure, the heartbeat is weak and irregular, or may even cease. Too great pressure causes a marked increase in amplitude and rate of beat. In this work, the perfusion rate was adjusted until the heart was beating steadily, and not showing more than slight passive swelling between beats. Sudden increases in the rate of perfusion produce an increase in the amplitude and rate of beat strikingly similar to that caused by the excitatory nerves. That this is not a reflex phenomenon involving these nerves can be shown by its continuance after complete removal of the central nervous system. On the other hand, a decrease in the pressure exerted within the heart by the perfusion fluid may cause slowing or cessation of beat. These results of internal pressure changes will be found helpful in explaining certain after-effects of nervous excitation and inhibition.

Effects of Faradic Stimulation of Cardio-Regulatory Nerves

Nervous inhibition

The inhibitory nerve to the heart was stimulated close to the basal portion of the recurrent cutaneous nerve, just forward of the ventral thoracic ganglion (see Fig. 1). With the ventral approach to the inhibitory nerves, there is little danger of confusion from accidental stimulation of the excitatory nerves. In this work, stimuli only slightly above threshold have been used, and in properly set up preparations no effects of any spread of current from the point of stimulation have been noted.

With inductorium stimulation, cardiac inhibition is usually complete, there being only a very narrow range in which partial inhibition can be obtained. For this reason, the inductorium is useful mainly to determine the intensity threshold for complete inhibition. The inhibition obtained by these means is an abrupt cessation of heartbeat when the intensity threshold is reached. This persists for ten to sixty seconds if the stimulus is continued, after which "escape" beats occur, leading to restoration of a normal beat. If the stimulus is discontinued while the heart is stopped, the beat is restored immediately, without perceptible inhibitory after-effect. In a few cases, the first beats after inhibition are of increased amplitude or rate. Observation has shown that during the period of inhibition, the continued inflow of fluid sometimes stretches the heart passively, as indicated by a rise in the traced record. When a stretching of the muscle occurs, the effect at the restoration of beat is the same as that resulting from an increase in perfusion rate, hence the heart responds by an augmentation of beat. In cases where the rate of perfusion is low, or when the heart stopped in diastole still tends to drain naturally, there is no evidence of distention, and as a result, no stimulatory after-effect of inhibition.

Nervous excitation

The two pairs[3] of excitatory nerves arise dorsal to the bases of the nerves to the third maxillipeds and the chelipeds respectively (see Fig. 1). Stimulation of these nerves in the vicinity of the heart is not effective because of interference from the nearby inhibitory fibers, but they are easily stimulated as they leave the thoracic ganglion. Commonly, the electrodes were placed to the rear of either of the large nerves mentioned above, dorsal to whose roots the heart nerves arise. However, because of the fact that the inhibitory nerves arise only slightly anterior to the excitatory, it is advisable to sever the inhibitory nerve on the side where the excitatory nerves are being stimulated. Excitation caused by faradic stimulation does not show a sharp threshold. It develops gradually with increasing intensity of stimulation, and soon reaches a maximum. One cannot well speak of "partial excitation." It would have been desirable to establish a quantitative measure of the amount of excitation produced, but because of the great variation in the character of the response in different hearts no reliable method was devised. According to the condition of the valves after the manipulation attending cannulation, the accelerated heart may literally pump itself dry, or it may retain fluid and increase the internal pressure. With these and possibly other variables operating to modify the picture of nervous augmentation of heartbeat, faradic stimulation of the excitatory nerves may produce an increase either in rate or amplitude, or in both. The after-effect of excitation is likewise variable, but in contrast to inhibition, excitation commonly shows a stimulatory after-effect, which in some cases lasts for several minutes after stimulation has ceased. This is most noticeable when a preparation is fresh.

A factor which may act to conceal a stimulatory after-effect, and which may even result in a depression, is the tendency of some hearts to pump themselves dry when accelerated, leading to temporary cessation of beat following the period of excitatory stimulation. Nevertheless, the number of times that excitation of the heart has resulted in an unquestionable after-effect in these experiments, as well as in those of earlier workers (Bottazzi, 1901; Conant and Clark, 1896), makes it appear that this phenomenon is a characteristic feature of nervous excitation of the crab heart, as a result of faradic stimulation.

That there is an upper limit to the effectiveness of the excitatory nerves is shown by their inability to produce a state of tetanic contraction in the heart, although the heart may readily be tetanized by direct stimulation, while acetylcholine

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[3] No excitation of the heart by stimulation of "Lemoine's nerve" has been noted in this work

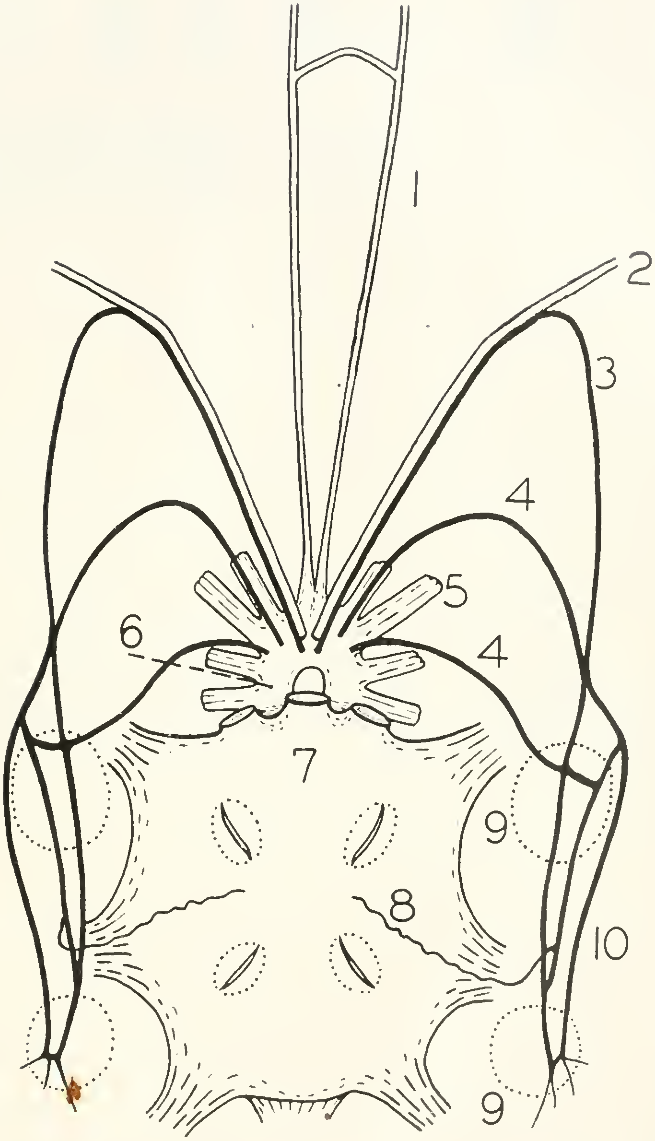

FIGURE 1. Schematic representation of the innervation of the heart of *Cancer* in dorsal aspect, with the thoracic ganglion shown as if moved forward slightly. Approximately to scale, but with cardiac nerves and pericardial plexus drawn with disproportionately heavy lines. (*1*) Circumoesophageal connective, (*2*) recurrent cutaneous nerve, (*3*) inhibitory cardiac nerve, (*4*) (*4*) excitatory cardiac nerves, (*5*) nerve to cheliped, (*6*) thoracic nerve mass, (*7*) heart, (*8*) dorsal nerve entering heart, (*9*) (*9*) branchio-cardiac orifices, (*10*) lateral pericardial plexus.

in high concentrations has been shown to cause an accelerated beat leading to a tetanus (Welsh, 1939a, b; Davenport, 1941).

Such results indicate that the extrinsic excitatory nerves do not directly control the rate of heartbeat, but must act to alter the rate of spontaneous activity in the pacemaker mechanism of the heart. Presumably the effectiveness of these nerves lies in their ability to alter the conditions determining the activity of the cardiac ganglion without being able to force the latter beyond certain limits.

It is commonly stated that the crustacean heart muscle, unlike the vertebrate heart, shows no absolute refractory period. This generalization holds when the heart is stimulated via the excitatory nerves, since cardiac excitation may commence at any phase of the beat, resulting in a well-marked summation. In this respect, the crustacean heart reacts rather more like skeletal muscle than like vertebrate cardiac muscle.

Effects of Frequency of Stimulation Upon the Cardio-Regulatory Nerves

In an effort to obtain a clearer picture of the action of the cardio-regulatory nerves, they were stimulated over a wide range of frequencies, employing a thyratron stimulator which delivered repetitive shocks over a range of 10 to 1,800 per second, with voltage adjustable from 0 to 10 volts. This stimulator could be connected at will to either of the two sets of electrodes in use, allowing comparable studies to be made on excitatory and inhibitory nerves in the same preparation over the same period. Both electrodes were fixed in place and undisturbed through an experiment.

Preliminary experiments showed that at very low or high frequencies the voltage required to produce a response was higher than at intermediate frequencies. Accordingly, a series of trials was made to determine the threshold voltage at various frequencies. Frequency-intensity threshold curves were plotted, showing the changes in intensity threshold over a wide range of stimulation frequencies, and the frequency limits above and below which stimulation of the heart nerves produced no effect. The results of a typical experiment are shown in Figure 2.

The greatest difficulty in this method is the problem of recognizing the onset of response in the heart at low or high frequencies outside of the optimum range. Over most of the frequency range, inhibition is total and begins abruptly, so that the threshold is easily determined, but at low frequencies, inhibition may first appear as a slight, gradually increased slowing of the heart, which may or may not cease beating abruptly when a higher voltage is reached. In such cases, two thresholds must be noted, one for partial, and one for total inhibition. In the case of excitation, while the onset of response is sharp over most of the frequency range, there may be a gradual acceleration at low frequencies that is especially hard to detect. It has not been possible to establish any criterion of "partial" excitation, hence the first noticeable increase in rate or amplitude has been taken as the threshold. Obviously, in all tests of this sort it is important to raise the intensity at a uniform rate, ceasing to raise the intensity as soon as results are observed on the kymograph tracing. Further to lessen the subjectivity of observations, tests were carried out in a planned series without stopping to verify individual readings. The series of frequencies were then passed through in the reverse direction in such a way that descending frequency settings alternated with those of the ascending series. No curves were con-

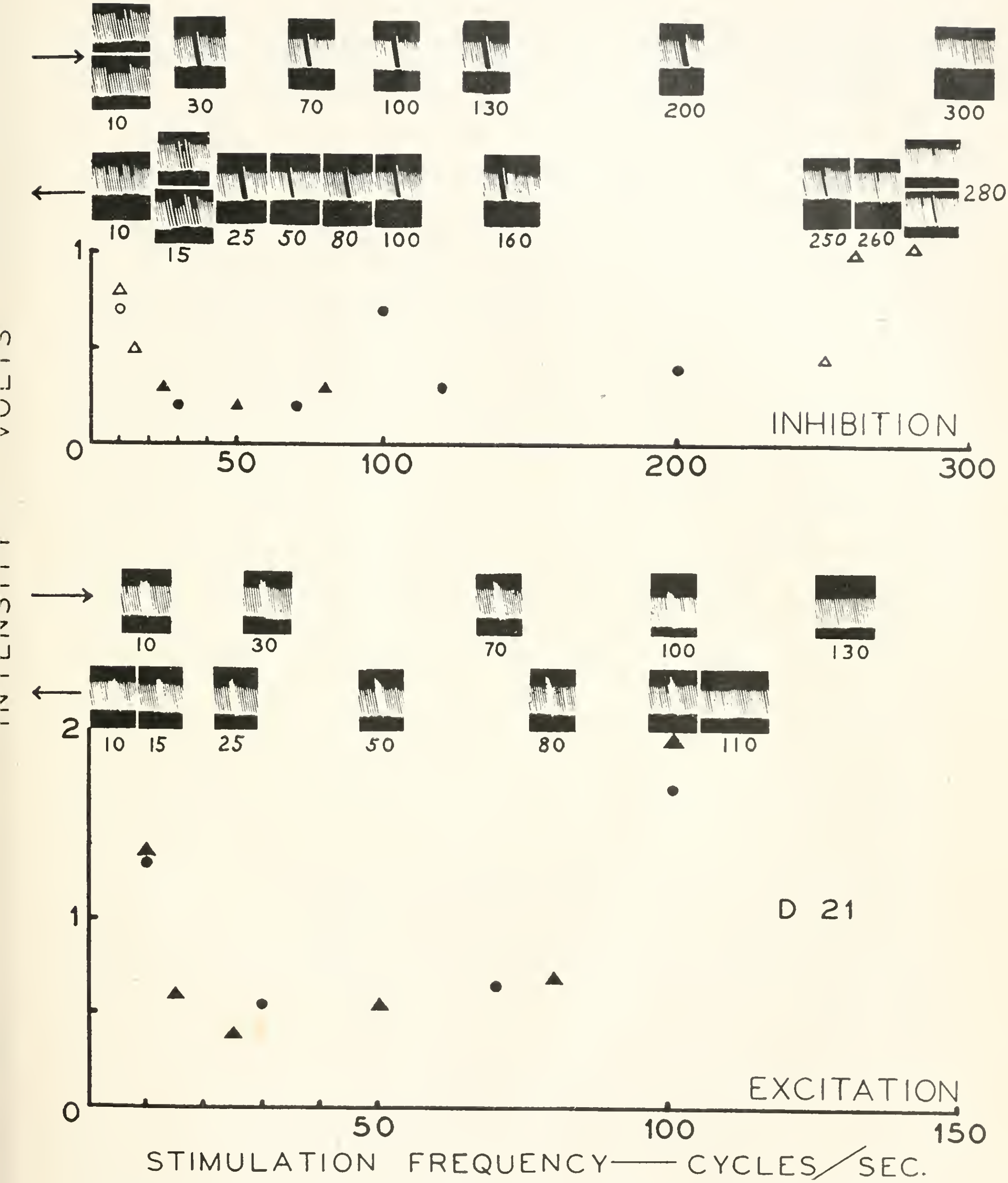

FIGURE 2. Frequency-intensity threshold curves for stimulation of the cardiac inhibitory and excitatory nerves, with tracing of the heart response at each frequency. Circles on the curve represent determinations made on an ascending scale of frequencies; triangles those on a following descending frequency series. Both curves obtained from the same animal (D–21), with tests made alternately on inhibitory and excitatory nerves. Open symbols indicate thresholds for partial inhibition at frequencies where no total inhibition could be obtained.

sidered valid if the electrodes had to be moved during the course of the experiment. Certain generalizations may be made regarding the effects of the frequency of stimulation on the cardio-regulatory nerves.

The excitatory nerves

1. The excitatory nerves show a low intensity (voltage) threshold for stimulation over a wide range of frequencies.

2. The lower frequency limit for effective stimulation of the excitatory nerves is lower than that for the inhibitory nerves. At frequencies as low as 10 per second there is often only a very slight rise in the threshold intensity of stimulation.

3. The upper frequency limit of effectiveness of stimulation of the excitatory nerves is variable, usually between 100 and 300 per second, but in some cases ranging well above 300 per second. In such cases it is unlikely that the nerve is actually discharging at such a high rate. The usual upper frequency limits are in agreement with the findings of Bogue and Rosenberg (1936), who report that in the leg nerves of *Maia* a large proportion of the motor fibers respond regularly to stimuli delivered at the rate of 200 per second, but that at 400 per second the fibers cease to respond in phase.

4. In the lower range of stimulation frequencies, higher voltages are required to stimulate the excitatory nerves, and less effect is produced upon the heart than when higher stimulation frequencies are employed. Raising the intensity of low-frequency stimulation does not produce the marked increase in response of the heart that can be produced by raising the frequency. This finding is consistent with the small number of nerve fibers innervating the heart and with the marked facilitation exhibited by crustacean nerves.

The inhibitory nerves

1. There is a frequency range from about 15–20 stimulating shocks per second up to 100 or 200 per second in which inhibition is complete at or near the threshold voltage of inhibitory nerve stimulation.

2. At a lower range of frequency, inhibition becomes only partially effective. In some preparations, stimulation of the inhibitory nerve at 10 per second has no visible effect on the heart, regardless of the stimulus intensity.

3. At the upper range of effective frequencies, stimulation of the inhibitory nerve often causes only partial inhibition. There is so much variation in the upper limit of effectiveness that no significance can be attached to its value in the individual preparation.

The effect of aging of the preparation upon the frequency-intensity threshold curves for inhibitory and excitatory nerves

Since in the experiments with perfused drugs, to be described below, it was necessary to run repeated series over periods of several hours, a number of undrugged preparations were tested repeatedly in order to determine the effects of fatigue. There is exhibited in successive runs a slight raising of the intensity threshold, associated with a narrowing of the frequency range over which stimulation of the heart nerves is effective. This is most marked at the upper end of the frequency range, where no certain significance can be attached to it. The lower end of the frequency curve shows much less tendency to shift with aging than does the upper. For this reason, in the experiments with drugs, the main attention was directed to that portion of the frequency curve lying below 100 per second.

Simultaneous Stimulation of Inhibitory and Excitatory Nerves

Using two inductoria, excitatory nerves have been stimulated during the course of nervous inhibition. In all cases where equal intensities have been used, inhibition remains dominant, and is not interrupted even when much stronger intensities of stimulation are applied to the excitatory nerve. Also, if an inhibitory nerve is stimulated during a period of nervous excitation, inhibition readily interrupts the excitation. But since a stronger inhibitory stimulus is required to stop a heart already under the influence of the excitatory nerve than is required to stop the unstimulated heart, it would seem that to a certain extent the inhibitory and excitatory nerves are each able to modify the effects produced by the other.

Further work, especially electrical studies of the ganglionic pacemaker within the heart, is needed before it can be said whether or not the "blending" of the actions of inhibitory and excitatory heart nerves means that these opposing nerves affect the same portion of the pacemaker system.

Effects of Strychnine upon Nervous Excitation and Inhibition of the Crab Heart

A number of tests have been made of the action of strychnine upon the cardiac inhibitory and excitatory nerves. Because strychnine in the concentrations necessary to affect the regulatory nerves has been found to affect the heartbeat adversely, about half of the experiments have been inconclusive. This is especially true in regard to studies on inhibition, since a heart that has stopped beating cannot be further inhibited, even though it can frequently be made to beat by stimulation of the excitatory nerves. The effects of strychnine on the character of the heartbeat generally follow a sequence that is passed through more or less quickly depending upon the concentration of drug applied. There is first a moderate increase in rate and amplitude of beat, giving way to a phase of grouped beats, in which groups of 2–8 beats are separated by short (2–5 second) pauses. With a decrease in the number of beats in a group, a state is reached in which the heart records single spike-like beats at intervals of 10–30 seconds. These beats commonly pass to the form of brief tetani, lasting some 1–4 seconds, which are a very characteristic result of strychnine in the crab. If poisoning is more severe or long continued, the heartbeats may become irregular or cease altogether, although retaining their amplitude to the last. The heart will usually recover if thoroughly washed.

The effects of strychnine upon nervous inhibition

Twelve experiments were performed, of which three were discarded because the electrodes had to be moved during the run, indicating poor setting or local injury to the nerve, while in three the heart failed before all the necessary tests could be carried out. The remaining six indicate that strychnine, in concentrations of about 1:2500 (4×10^{-4}), is able to abolish the effect of the inhibitory nerves after perfusion for 30 to 60 minutes. The loss of response was verified in all cases by decreasing the coil distance of the stimulating inductorium by 5 mm. After a short period of washing, during which the beat usually showed a temporary depression, the inhibitory effect of the nerve was restored to or nearly to the initial value; in all cases the threshold after restoration was lower than the strongest stimulus used to verify the abolition of inhibition. In each of two experiments the abolition of inhibition in strychnine and its restoration after washing were repeated four times.

The effects of strychnine upon nervous excitation

The records of the effects of strychnine upon the effectiveness of the excitatory nerves to the heart are somewhat more reliable than are those for inhibition, since excitatory effects may frequently be obtained even after the heart has ceased to beat. Strychnine in concentrations of 1:2500 (4×10^{-4}) blocks the action of the excitatory heart-nerves, and this effect is fully reversible.

These experiments indicate that the cardiac regulatory nerves can be paralyzed by strychnine, as the somatic motor nerves presumably are, without causing the heart to cease beating. However, the anomalous character of the beat in the strychninized heart indicates that the intrinsic pacemaker neurones are affected considerably, though perhaps to a lesser degree than are the regulators.

Effects of Atropine Upon Nervous Inhibition and Excitation

Methods of study involving the use of inductorium stimulation, as in the work on the effects of strychnine described above, failed to reveal any effect of atropine on either the excitatory or the inhibitory nerves. Accordingly, confirmatory studies were made by the method of variable-frequency stimulation. Frequency-intensity threshold curves have been determined before and during perfusion with atropine, as well as after a period of washing.

The effects of atropine upon nervous inhibition

Atropine sulphate was perfused in concentrations of 10^{-5} and 10^{-4} for periods of 15–30 minutes before determining the frequency-intensity curves for the inhibitory nerves while the heart was still being perfused with the drug. The application of the drug followed, of course, the determination of such a curve while the heart was bathed with perfusion fluid. The cardio-inhibitory nerves of *Cancer* are not blocked by atropine, but are actually slightly augmented in their effects.

The effects of atropine upon nervous excitation

Atropine sulphate was perfused in concentrations of 10^{-6} to 10^{-4} for 15–30 minutes, as in the preceding experiments. The frequency-intensity threshold curves indicate that atropine in these concentrations has no significant effect upon the cardiac excitatory nerves. That a sufficient concentration of atropine (10^{-4}) was used is indicated by the fact that Welsh (1939b) found that atropine 10^{-5}

would largely block the effects of acetylcholine on the isolated heart of *Panulirus*, while atropine 10^{-4} is effective in this respect on the heart of *Astacus* (Davenport, Loomis, and Opler, 1940) and atropine 5×10^{-5} on the isolated heart of *Cancer magister* (Davenport, 1941).

EFFECTS OF NICOTINE UPON NERVOUS INHIBITION AND EXCITATION

In view of the failure to block nervous inhibition and excitation with atropine, it was of interest to see how nicotine might affect these processes, since certain effects of acetylcholine ("nicotine-like" effects) are not abolished by atropine. A number of experiments were performed, using faradic stimulation. The results have been checked by variable-frequency stimulation. Nicotine, when first applied, has a strong augmenting effect on amplitude and rate of beat. The stimulatory effects wear off after a time, and, if the concentration of nicotine is high, depression of heartbeat ensues. Irregularity and grouping of beats are common in the early stages of nicotine depression; later stages are marked by infrequent beats or by an abnormal condition of intermittent tetanus. This last type of beat may be the result of a direct response of the heart muscle to stretching, as it could be seen in the records that the heart swelled noticeably between "beats." The crab heart adapts to nicotine, hence successive increases in the strength of drug applied produce relatively small effects.

The return of the heart to perfusion fluid after being in a strong nicotine solution frequently results in a temporary cessation of beat, following which the beat may be greatly accelerated as the nicotine concentration falls from a depressant to an excitatory level. During the period of complete stoppage, the excitatory nerve is able to function, an indication that this nerve is more resistant to the paralyzing effects of nicotine than is the cardiac ganglion.

The effects of nicotine upon nervous inhibition

In no case has inhibition been abolished by nicotine in concentrations of 1 : 10,000 to 1 : 1,000. Hearts responding to freshly applied nicotine by a marked frequency increase can be inhibited as readily as the normal heart, or as hearts which have been greatly depressed by strong concentrations of nicotine. The action of the inhibitory nerves can be studied only as long as the heart continues to beat, hence the effect of dosages of nicotine of strengths greater than 1 : 1,000 could not be determined.

The effects of nicotine upon nervous excitation

Nicotine in strengths of 1 : 1,000,000 to 1 : 500 has been tested, with inductorium stimulation of the excitatory nerves. The results fall into three classes, depending upon the strength of drug used and its effect on the rate of heartbeat. Nicotine in the lower concentrations (10^{-6}), or when first applied, has a marked positive chronotropic effect which may persist for some time. In certain experiments, stimulation of the excitatory nerve during this period of excitation has shown an augmentation of the effectiveness of the nerve as compared to its effectiveness upon the undrugged heart.

In intermediate concentrations of nicotine (10^{-5}), or in concentrations applied for a length of time sufficient to depress the heartbeat, nervous excitation is not impaired. This fact indicates that the cardiac ganglion is more sensitive to nicotine depression than are the excitatory nerves. The complete stoppage of heartbeat that may follow the return of a heart to perfusion fluid after being in nicotine supports this view, since the excitatory nerves may stimulate a heart that has ceased its spontaneous beat under such conditions. In this situation the pacemaker ganglion has evidently lost its capacity for spontaneous discharge, although it must still be able to transmit impulses to the heart muscle via the neuromuscular junctions when properly stimulated. In considering the effects of nicotine, it must be kept in mind that the drug may not only block transmission at the nerve endings of the ganglion and extrinsic nerves, but may act on the ganglion cell bodies themselves, causing a loss of their rhythmical activity.

In high concentrations of nicotine the effects vary, depending upon whether the heart gives a slow beat of normal form or whether it passes into a state of irregular intermittent tetani. In the former case, stimulation of the excitatory nerve may still be able to rouse the heart to more rapid beating, but in the latter case the excitatory nerve appears to be ineffective.

Frequency-intensity threshold curves for the excitatory nerves showed no significant changes after perfusion of hearts with nicotine 10^{-6} or 10^{-5}. Higher concentrations of nicotine could not satisfactorily be tested in this way, since the hearts generally failed during the lengthy process of determining the curve. Thus even though there is evidence that certain concentrations of nicotine enhance the response to excitatory nerve stimulation, it has not been possible to show any increased effectiveness of lower rates of excitatory nerve stimulation.

The augmentation of excitatory nerve effectiveness by low concentrations of nicotine may perhaps be comparable to the action of nicotine on vertebrate autonomic ganglia, where low concentrations of the drug augment the effect of preganglionic stimulation. In high concentrations, nicotine blocks the passage of impulses at vertebrate autonomic ganglia. In the crab heart nervous excitation has been blocked by nicotine in high concentrations, but only in cases where the heartbeat is of abnormal form. Is this failure of excitation due to a blocking of the excitatory nerve, or is it due to overstimulation of the heart ganglion? The abnormal form of the heartbeat suggests that the function of the heart ganglion has been impaired. If we accept this explanation, we cannot say whether or not transmission at the endings of the excitatory nerves is being blocked.

To sum up the results of this study of the effects of nicotine on the cardio-excitatory nerves, low concentrations of nicotine augment the action of these nerves, and high concentrations appear to block their effects. But, because of the likelihood that the heart ganglion is at least as sensitive to nicotine as are the excitatory nerves, it is not possible to say whether the block occurs at the endings of the excitatory nerves themselves or in the heart ganglion. The evidence so far obtained points to the presence of a "nicotine-like" cholinergic mechanism involved in nervous excitation of the crab heart, but it does not yet allow us to decide between the cardio-excitatory nerves and the intrinsic heart ganglion as possible sites of this mechanism. Perhaps both possibilities are true, since any effects of nicotine observed could be explained on such a basis. The methods employed in this work do not enable us to distinguish between nicotine-block of the cardiac ganglion alone and simultaneous nicotine-block of the excitatory nerve and the heart ganglion.

Effects of Eserine Upon Nervous Excitation and Inhibition

In the crabs used in the present work, eserine (physostigmine sulphate Merck) in concentrations of 10^{-6} or 10^{-5} was found to potentiate markedly the effects of low concentrations of acetylcholine. Hence such concentrations of eserine were considered sufficient to inactivate a significant amount of the cholinesterase in the heart when perfused for 15 minutes or more. Such low concentrations of the drug do not, by themselves, visibly affect the heartbeat.

The effects of eserine on nervous inhibition

Eserine in concentrations of 10^{-6} to 4×10^{-5} did not augment the action of the cardio-inhibitory nerves when tested by inductorium stimulation, thus agreeing with results previously obtained on *Panulirus* (Smith, 1940–41), nor could any effect of eserine upon frequency-intensity threshold curves be detected.

The effects of eserine on nervous excitation

Since the effects of nicotine on cardio-excitation have made it appear at least possible that the excitatory nerves are cholinergic, the action of eserine on the effectiveness of these nerves is of importance. However, no success was obtained in producing an augmentation of excitation by the application of eserine. Inductorium stimulation did not produce detectable results, while frequency-intensity threshold curves show little more. Eserine either has no effect, or it actually makes cardio-excitation more difficult to obtain. It should be recalled that the concentration of eserine used was sufficient to augment the action of acetylcholine on the crab heart, but that it did not by itself cause any stimulation of the heart. Under such conditions, Wiersma and Novitski (1942) have found eserine to augment the the effects of nervous excitation of the crayfish heart, as determined by a study of heart rate.

Effects of Acetylcholine Plus Eserine Upon Nervous Excitation and Inhibition

It has been found that a low concentration of acetylcholine chloride (10^{-7}) has a long-continued stimulatory action upon the heart of *Cancer* if it is potentiated with eserine 10^{-5}. Neither drug has any marked or persistent effect if perfused alone. Such a mixture of drugs has been used to determine the effects of acetylcholine upon the action of the regulatory nerves. But as in the case of erserine alone, "protected" acetylcholine in low, stimulatory concentrations has no consistent or significant effect either upon the inhibitory nerves or upon the excitatory nerves. Because higher concentrations of acetylcholine gave effects too strong to be maintained over the period necessary for the type of observations made in this study, they were not used.

Discussion

The lack of effect of eserine and acetylcholine upon the excitatory nerves would seem to speak against the possibility that these nerves are cholinergic. Yet there are indications that the failure to detect eserine potentiation of the effectiveness of these nerves is not conclusive evidence of their non-cholinergic nature. Welsh (1940–41) has reported that while eserine augments the effects of low concentrations of acetylcholine on the isolated lobster heart, it fails to potentiate, or even lessens the effects of acetylcholine stronger than 10^{-5}. It is probable that cholinergic nerves release at their terminations acetylcholine in small amounts but in high, sharply localized concentrations; while cholinesterase has been shown to be greatly concentrated at the motor end plates in vertebrate skeletal muscle (Marnay and Nachmansohn, 1938; Nachmansohn, 1939). In the case of vertebrate autonomic ganglia, Brown and Feldberg (1936) have shown that eserine can potentiate the effect of preganglionic stimulation so effectively that the ganglion cells are soon paralyzed by the accumulation of acetylcholine unless submaximal preganglionic volleys and low frequencies of stimulation are employed. If such a situation exists in the crab, it is possible that eserine may allow the accumulation of mediator substance in depressant concentrations. In view of the evidence of Wiersma and Novitski (1942) that eserine augments nervous excitation of the crayfish heart, a restudy of this matter in the crab would be desirable, employing a technique by which heart rate, uncomplicated by amplitude changes, could be observed.

The inability of the excitatory nerves to tetanize the crab heart, although this state may be produced by direct electrical stimulation, acetylcholine, nicotine, etc., raises the question of whether or not the excitatory nerves act in the same way or at the same point as these other agents. Prosser (1943) has shown that the systole of the neurogenic heart of *Limulus* is initiated by synchronous activity in the cardiac ganglion, while tetanus results from a continued asynchronous discharge. During the course of the present work it has been observed that crab hearts depressed by nicotine or strychnine often exhibit a series of very irregular abnormal beats which, during recovery, regroup themselves to normal systoles, apparently by a return of the several cardiac ganglion cells to synchronous activity. The short tetani that may replace the normal systoles under the influence of nicotine or strychnine may, likewise, possibly be caused by asynchronous discharges of ganglionic cells. Direct electrical stimulation, acetylcholine, and other agents capable of tetanizing the neurogenic arthropod heart may do so by throwing the cardiac ganglion into a state of asynchrony, but it would seem that the excitatory nerves are capable of producing only more closely spaced bursts of synchronous ganglionic activity.

Since other evidence so far accumulated supports the view that the heart ganglion is cholinergic, and since the lack of effect of atropine upon normal heartbeat indicates that the ganglion does not exert a "muscarine-like" action on the heart, muscle, we may consider the possibility that the ganglion has a "nicotine-like" effect at the neuromuscular junction. It will be recalled that, in its action on different vertebrate tissues, acetylcholine has a variety of effects, which may be broadly classified into "muscarine-like" effects that can be blocked by atropine, and "nicotine-like" effects that can be blocked by high concentrations of nicotine. These classifications of acetylcholine action apply, strictly, to the reactions of vertebrate tissues, and it is possible that the reactions of invertebrate tissues do not fall into identical categories. Hence, the fact that the crustacean heart ganglion does not appear to exert a "muscarine-like" action upon muscle does not prove by elimination that its effects are fully comparable to the "nicotine-like" action of certain cholinergic nerves in vertebrates. For the present, however, we may consider that the cardiac ganglion transmits impulses to the heart muscle by a cholinergic mechanism having a somewhat "nicotine-like" effect.

If it is true that both the ganglion and the excitatory nerves are of the same pharmacological nature, this fact renders useless our attempts to demonstrate a nicotine block of the excitatory nerves. As pointed out above, the ganglion seems more sensitive to nicotine than are the excitatory nerves. Paralysis of the ganglion removes an essential link in the chain of impulses which pass from regulatory nerve to heart muscle in nervous excitation. With the ganglion out of action, the present methods do not reveal whether or not the excitatory nerves are blocked by high concentrations of nicotine, but the positive evidence so far obtained points to a cholinergic "nicotine-like" action of the cardio-excitatory nerves upon the heart ganglion.

Summary

1. The heart of *Cancer irroratus* has been perfused *in situ*, the beat recorded mechanically, and the inhibitory and excitatory nerves stimulated while the heart was perfused with various drugs.
2. Nervous inhibition of the heart induced by stimulation of the inhibitory nerves is usually total with no inhibitory after-effect.
3. Nervous excitation of the heart induced by stimulation of the excitatory nerves frequently shows a stimulatory after-effect.
4. The cardio-inhibitory nerves appear to be more effective than the excitatory nerves, but each type of nerve can modify to some extent the effectiveness of the other.
5. The excitatory nerves are effective when stimulated at frequencies ranging from 200–300 per second down to 10 per second or less.
6. The inhibitory nerves produce total inhibition when stimulated from 15–20 to 100–200 times per second. At frequencies extending above and below this range, partial inhibition may be obtained.
7. Strychnine, in concentrations of 1 : 2,500, blocks reversibly the action of both excitatory and inhibitory nerves.
8. Atropine is without effect upon excitatory and inhibitory nerves, as well as upon the heart ganglion in concentrations sufficient to abolish the action of applied acetylcholine.
9. Nicotine, in low concentrations, augments the effectiveness of the excitatory nerves as well as of the heart ganglion.
10. Nicotine in high concentrations may block the passage of impulses from cardiac ganglion to muscle; hence it could not be determined with certainty if nicotine blocks the excitatory nerves.
11. Neither eserine alone, nor with acetylcholine in stimulatory concentrations, augmented cardio-inhibition or excitation. It is suggested that the lack of effect of eserine upon the excitatory nerves deserves restudy.
12. It is probable that the excitatory nerves are cholinergic, and exert a somewhat "nicotine-like" effect upon the heart ganglion, which in turn exerts a "nicotine-like" action at the neuromuscular junction.
13. No light has been thrown on the pharmacological nature of the cardio-inhibitory nerves.

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PHYSIOLOGY OF INSECT DIAPAUSE. II. INTERACTION BETWEEN THE PUPAL BRAIN AND PROTHORACIC GLANDS IN THE METAMORPHOSIS OF THE GIANT SILKWORM, PLATYSAMIA CECROPIA

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In the Cecropia silkworm the progress of metamorphosis is interrupted as soon as the pupa is formed. There then intervenes a prolonged period of pupal diapause characterized by cessation of growth and differentiation. The mechanism that converts this cellular dormancy of diapause into the intense activity of adult formation was examined in a previous investigation performed on several species of giant silkworms (Williams, 1946b). For these species it was evident that diapause is under the control of the insect's brain. This control consists in a dependency of adult development on the action of a factor arising from the brain; the brain, in turn, is rendered competent to release this factor by exposure to low temperatures. It is the purpose of the present report to describe in greater detail the nature of the activating mechanism.

Materials and Methods

The present communication is based on a total of 282 experiments performed, for the most part, on pupae of the giant silkworm, *Platysamia cecropia*. In a few experiments pupae of related genera were studied, including *Telea polyphemus, Callosamia promethea,* and *Samia walkeri*. The management of this array of animals was essentially identical to that described previously (Williams, 1946b).

Direct or Indirect Action of the Brain?

If the brain is removed from a diapausing pupa, the resulting insect never develops further and persists until death in permanent diapause—a matter of two years in some individuals. Yet at any time during this period adult development can be evoked by implanting into the brainless pupa a brain obtained from a previously chilled pupa.

It was a rational assumption that the developmental factor from the implanted brain had some direct action on the host tissues whereby the latter were converted from dormancy to activity. Yet the possibility remained that the brain's action might be indirect rather than direct, in a sense familiar to endocrinologists, as, for

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example, in the action of certain of the pituitary hormones on the uterus. Experiments have therefore been performed to test these two possibilities.

To this end techniques have been developed for subdividing individual pupae into fragments. In my experience division of these stout animals by means of ligatures has not met with success. Nor did gross slicing of the pupae into parts, as practiced by Crampton (1899), Hirschler (1903, 1904), and Hachlow (1931), produce viable preparations. A simple, successful technique that was ultimately developed will be described briefly.

Isolation of Pupal Fragments

The pupa, under continuous carbon dioxide anesthesia (Williams, 1946a), is placed on its side and the abdominal cuticle plus underlying hypodermis incised around the circumference of the abdomen at the level of the tips of the wingflaps. The incision is confined to the thin intersegmental membrane. Further maneuvers are designed to separate the abdomen from the anterior fragment without rupturing the midgut.[2] The intersegmental muscle masses, the heart, and the nerve cord are, in turn, cut through. The fat body and hindgut are then transected and the attachments of tracheae swept away from the walls of the midgut. The midgut plus nearly all of the Malpighian tubules may now be placed in the anterior fragment. Or by further dissection the midgut may be removed from the anterior fragment and discarded.

The cut surface of each of the two fragments is then sealed by melted paraffin to a circular, plastic cover slip which is provided with a centrally placed hole. Through the hole insect Ringer's is added to displace all air and the hole is finally plugged with melted paraffin.

It may be noted that these isolated abdomens (Fig. 3) consisted, in reality, of the terminal six abdominal segments, the anterior four abdominal segments being carried away with the anterior end (Fig. 1). Transections at other levels were occasionally accomplished, but the one described proved to be most favorable. In

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[2] Rupture of the midgut floods the body cavity with a dark green fluid that contains a good deal of particulate matter and shows a broad, dense absorption band centering at 670 mμ. The fluid is apparently non-toxic, but the particulate matter is frequently drawn into the cut end of the heart. Within the heart it then acts as embolus to occlude the aorta at its narrowest portion just behind the brain. Such animals usually fail to survive.

Explanation of Plate I

Approximately Life Size

Figure 1. Anterior fragment of a brainless, diapausing pupa.

Figure 2. Fragment in Figure 1, after adult formation. Development was evoked by implanting a previously chilled brain.

Figure 3. Posterior fragment of a diapausing pupa.

Figure 4. Fragment in Figure 3, after adult formation. Development was evoked by implanting a previously chilled brain plus two pairs of diapausing prothoracic glands.

Figure 5. Posterior fragment of a diapausing pupa, showing critical level of section that includes the meso- and metathorax.

Figure 6. Fragment in Figure 5, after adult formation. Development was evoked by implanting a previously chilled brain. Note the complete development of those parts of the antennae, legs, and wings that were present in the fragment.

Plate I

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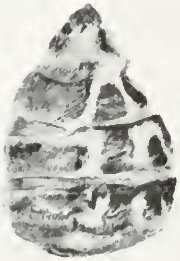

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certain instances abdomens were transected a second time to yield viable pairs of abdominal segments sandwiched between plastic slips.

The inner surface of the plastic slips is rapidly coated by a thin, transparent tissue formed by anastomoses developed between blood cells and, within about two weeks, by the outgrowth of epithelium and of tracheoles. At any time, however, one can operate inside the insect fragment by removing the paraffin plug from the centrally placed hole.

Of the entire series of isolated pupal parts, approximately two-thirds died within the first ten days after preparation. Death was invariably preceded by a darkening of the blood, a reaction which in itself seems to be toxic. The remaining preparations survived for considerable lengths of time, as indicated by the beating of the heart and by spontaneous movements of the abdominal segments. Isolated abdomens remained alive at 25° C. for up to eight months. Anterior ends survived for not more than two or three months, however, unless Ringer's solution was occasionally added to compensate for loss of water by evaporation.

Brain Implantations Into Pupal Fragments

Brainless diapausing pupae were transected at the level of the wing-tips to obtain ten pairs of viable anterior and posterior halves. Into each of these subdivisions a chilled Cecropia brain was then implanted (Fig. 1 and 3). Each anterior fragment proceeded to develop normally into the corresponding anterior end of a lively adult moth, the cut surface being closed by scaleless, regenerate, "chitinized" epithelium (Fig. 2). The isolated abdomens, to the contrary, remained undeveloped after brain implantation, although they continued to live for an average of three months.

With slight variations in technique these findings have been confirmed during the past three years on a total of 60 additional viable preparations. As many as six chilled brains have been implanted into a single abdomen without inducing development, even though some of these abdomens survived as long as eight months thereafter.

Explanation of Plate II

Approximately Life Size

Figure 7. Posterior fragment of a diapausing pupa joined directly to the tip of a brainless, diapausing pupa.

Figure 8. Preparation in Figure 7, after adult formation of host and graft. Development was evoked by implanting a previously chilled brain into the host. Note the regenerate tissue passing through the hole in the plastic slip to connect the two parts.

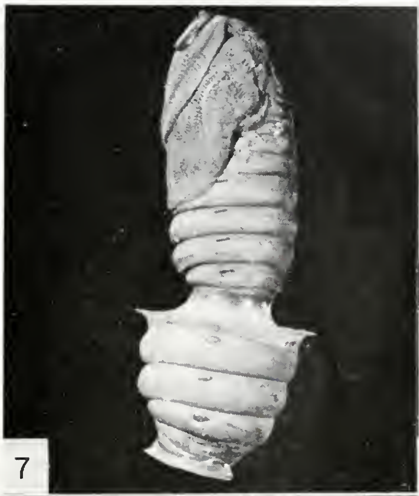
Figure 9. Posterior fragment of a diapausing pupa joined directly to the thoracic tergum of a brainless, diapausing pupa.

Figure 10. Preparation in Figure 9, after adult formation of host and graft. Development was evoked by implanting a previously chilled brain into the tip of the graft.

Figure 11. Posterior fragment of a diapausing pupa joined to a brainless, diapausing pupa by means of a short, square, plastic tube. Note the tube of regenerate tissue traversing the plastic tube's lumen. In order to follow the onset and progress of development, plastic windows have been placed at each end of the preparation.

Figure 12. Posterior fragment of a diapausing pupa joined to an anterior fragment of a brainless, diapausing pupa by means of a long, plastic tube. Note the tube of regenerate tissue within the plastic tube.

Plate II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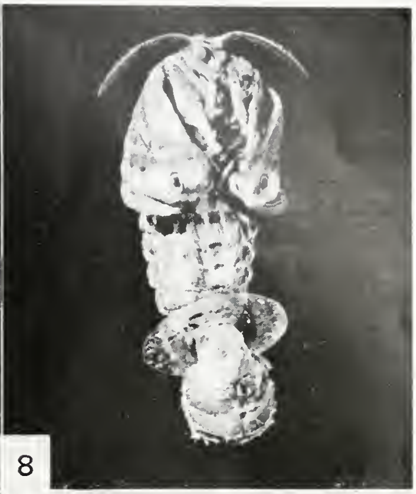
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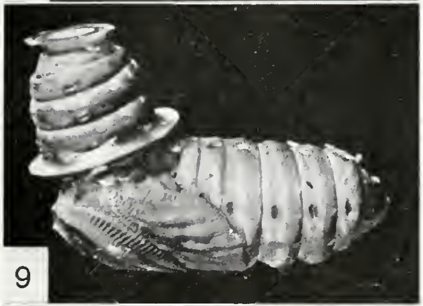
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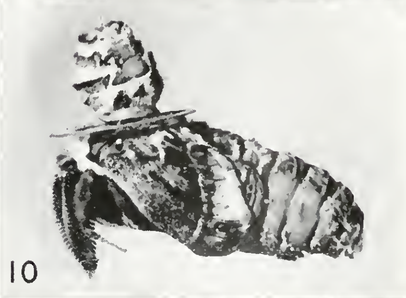
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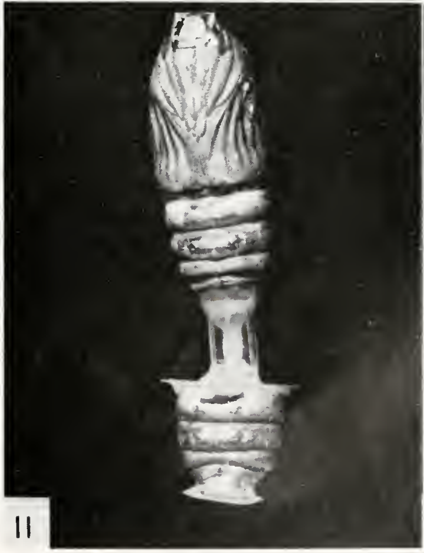
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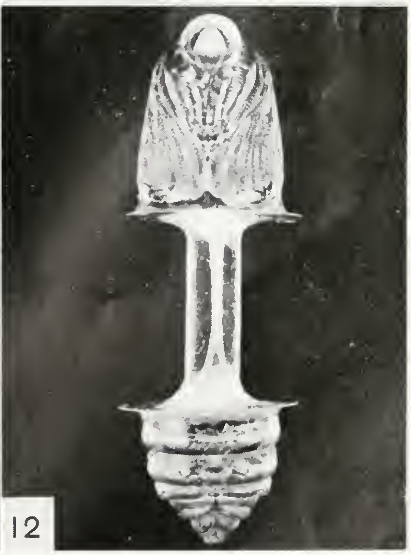
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It is therefore apparent that anterior and posterior fragments respond differently to brain implantation. This difference in response might be explained if the anterior fragment possessed a second developmental center that was lacking in the posterior fragment. To test this possibility the level of transection was varied.

TRANSECTIONS OF PUPAE AT VARIOUS LEVELS

Brainless, diapausing pupae were transected at various levels and the developmental capacity of the posterior fragments tested by implanting a chilled brain. The results of these experiments confirmed the findings of previous investigators that a "differentiation center" was present in the region of the thorax of lepidopterous pupae (Hachlow, 1931; Bodenstein, 1938; Bounhiol, 1938). Thus, abdomens in continuity with a pupal thorax invariably developed when a chilled brain was implanted. In contrast, similar treatment failed to evoke development of abdomens separated from the entire thorax. The critical level seemed to lie in the region of the mesothorax, for, after transection at this point, brain implantation induced development only after a long latent period of about six months (Fig. 5 and 6).

GRAFTING EXPERIMENTS ON ISOLATED ABDOMENS

The importance of this anterior differentiation center was further revealed in experiments in which isolated abdomens were grafted to brainless, diapausing pupae (Fig. 7, 9, and 11) or to the anterior fragment of brainless, diapausing pupae (Fig. 12). In such combinations, the host and the graft grew together to establish tissue continuity, but did not develop further. This continuity was developed even when plastic tubes, as long as 3 cm., were interposed between the two parts (Fig. 11 and 12).

Now, when a brain from a chilled Cecropia pupa was implanted into either host or graft, diapause was terminated and the entire preparation developed into the fully formed, corresponding parts of the adult (Fig. 8 and 10).

IMPLANTATION OF PROTHORACIC GLANDS

The evidence, up to this point, indicates that adult development after pupal diapause requires a factor from the brain plus some additional factor from the thorax. An extensive series of experiments was then performed in which a chilled brain plus various thoracic organs were implanted into isolated abdomens.

This search was vastly aided by the publication of Fukuda's paper in 1941. In this communication Fukuda describes an endocrine activity of the "prothoracic glands" in evoking development of ligatured pupal abdomens of the commercial silkworm, *Bombyx mori*.

The great significance of the prothoracic glands was confirmed. Thus, isolated abdomens of diapausing Cecropia pupae developed readily when provided with a chilled brain plus two pairs of prothoracic glands.[3] The pupal cuticle became crisp

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[3] Due to the numerous ramifications of the pupal prothoracic glands in the pro- and mesothorax, it was usually impossible to remove these organs in their entirety. For this reason two pairs were usually implanted into each isolated abdomen. This proved to be an important detail, for a single pair of the incomplete glands did not generally suffice to produce development after brain implantation. For a description of the prothoracic glands in *Platysamia cecropia* see Williams, 1948.

and was delaminated and the abdomens appeared as the lively, corresponding segments of the adult, the cut surface being closed by scaleless, regenerate, "chitinized" epithelium (Fig. 4). Development was complete externally and internally and, in the case of female abdomens, the eggs were matured. Similarly, abdomens isolated from chilled pupae after return to room temperature required both a chilled brain and prothoracic glands for the initiation of adult development.

In these experiments it was found that the prothoracic glands as well as the brain show a lack of species—or genus—specificity, for both of these organs remain effective when interchanged between *Platysamia cecropia* and *Telea polyphemus.*

In contrast to the results of Fukuda (1941), who studied a species without pupal diapause, no development of isolated abdomens occurred when prothoracic glands were implanted in the absence of a chilled brain.

Further Experiments on Isolated Pupal Abdomens

From a series of six isolated abdomens of diapausing Cecropia pupae there were further removed the gonads, the entire digestive tract, the Malpighian tubules (with the exception of a few loose fragments), and the entire central nervous system, including the residual chain of five ganglia and connectives. Three of these preparations survived, the heart continuing to beat. After the implantation of a chilled brain plus prothoracic glands, two of the abdomens developed into flaccid, but fully mature adult abdomens.

Discussion

It is evident from these experiments that the termination of pupal diapause requires a minimum of two factors, one from the brain and the other from the prothoracic glands. The easiest way to render these organs functional is to expose the diapausing pupa to low temperatures and then return it to room temperature. As has been shown previously, the effectiveness of chilling in terminating dormancy can be explained in terms of an action of low temperature on the brain, whereby the latter is made competent to release its developmental factor (Williams, 1946b). Manifestly, the prothoracic glands do not require similar exposure to cold, for they are promptly activated when a chilled brain is implanted into a diapausing pupa.

From these observations it may be concluded that the brain exerts a controlling action on the prothoracic glands. Subsequent experiments have confirmed this hypothesis consistently. Thus to induce the development of isolated pupal abdomens, it is necessary that the implanted brain be obtained from a previously chilled pupa. This is not the case in regard to the implants of prothoracic glands, for these organs are equally effective when obtained from diapausing pupae. The functional failure of the prothoracic glands at the outset of diapause seems therefore to result from a primary failure of the brain in releasing its factor.

The mechanism that terminates diapause must ultimately supply the dormant tissues with something necessary for cellular growth and differentiation. In respect to this process the experimental results may be interpreted from two points of view. On the one hand, the brain factor may be conceived as having sole action on the prothoracic glands and the prothoracic gland factor, in turn, as having specific effect on the tissues of the body in general. On the other hand, the tissues may require interaction with both factors, the first factor serving to condition the tissues for reaction with the second.

Although the evidence at hand does not suffice to exclude either interpretation,[4] it seems likely that the factor from the prothoracic glands has ultimate action on the dormant tissues to convert them to activity. Thus, we have noted previously that adult formation becomes independent of brain action as soon as the earliest sign of adult development is evident (Williams, 1946b). However, for some days thereafter, the animal continues to require further function of its prothoracic glands, for if the abdomen is isolated during this period, it ceases to develop. When tested in this fashion, adult development shows a dependency on the prothoracic glands until a critical stage, signalled by the initiation of eye pigmentation.

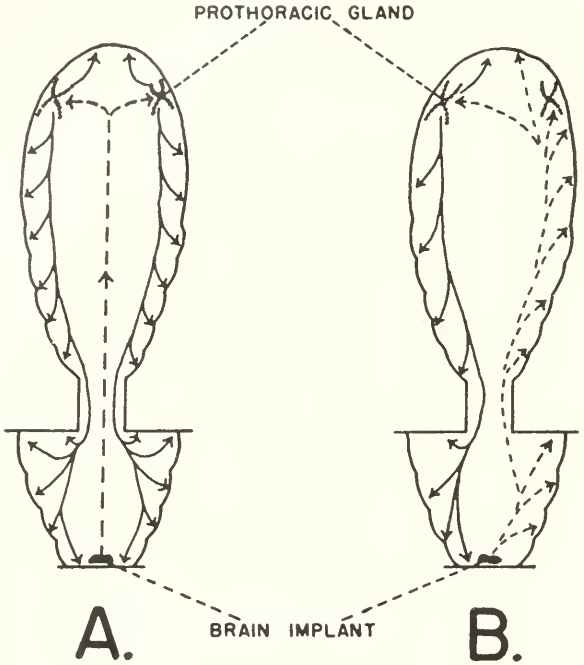

FIGURE 13. Diagrammatic interpretation of experimental results when a chilled brain is implanted into the tip of the graft in preparations such as Figure 11. Development begins first in the anterior end of the pupa, containing the prothoracic glands. For explanation, see text.

Further evidence favoring the view that the factor from prothoracic glands has ultimate action on the tissues has been derived from the experiments, described above, in which isolated abdomens were grafted to brainless, diapausing pupae (Fig. 7 and 9). When development was evoked by implanting a chilled brain *into the tip of the graft,* the brainless pupa, containing the prothoracic glands, was observed to initiate development one day in advance of the abdomen, containing the brain. This difference in time can be magnified to three days or longer by interposing a plastic tube between host and graft (Fig. 11). In such preparations the final organ to initiate development was the imaginal disc of the graft's genitalia, notwithstanding the fact that this lay alongside the implanted brain that had touched off the whole process.

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[4] Since this paper went to press, proof has been obtained of the validity of the first interpretation (see Fig. 13*a*).

In terms of this type of preparation we may finally summarize our present information diagrammatically, as indicated in Figure 13. In Figure 13*a*, the brain factor is viewed as activating the prothoracic glands and the prothoracic glands as activating the tissues. In Figure 13*b*, the brain factor is conceived to act on all the bodily tissues to prepare them for final reaction with the factor from the prothoracic glands. In either case, the brain is the organ of primary control, but this control, at least in part, is exercised by an indirect mechanism.

SUMMARY

1. The mechanism that initiates adult development after pupal diapause has been studied in a total of 282 experiments, supplementary to those reported previously.

2. Brainless, diapausing pupae were divided transversely and the developmental capacities of anterior and posterior fragments tested and compared.

3. Implantation of a previously chilled brain sufficed to terminate the dormancy of anterior fragments.

4. Isolated abdomens, to the contrary, remained undeveloped after brain implantation. Yet such abdomens, even without implantation, could be induced to develop by grafting them to developing anterior fragments. Manifestly, the abdomens required for development an additional factor normally produced in the anterior end of the pupa.

5. By transections at various levels the source of this additional factor was found to be the thorax.

6. A testing of various thoracic organs revealed the effectiveness of the "prothoracic glands." Thus, implantation of a chilled brain plus prothoracic glands induced the complete adult development of isolated abdomens. In this effect the prothoracic glands as well as the brain showed a lack of species—or genus—specificity.

7. The termination of diapause requires in these species the action of a minimum of two factors, one arising from the brain and the other from the prothoracic glands. The brain factor is necessary for the activation of the prothoracic glands.

8. The factor from the prothoracic glands, most probably, has ultimate action on the tissues in terminating diapause.

9. The brain is the organ of primary control over diapause in the species studied, but this control, at least in part, is exercised by an indirect mechanism.

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A COMPARATIVE STUDY OF THE LIPIDS IN SOME MARINE ANNELIDS

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Introduction

In the past, studies on the metabolism of lipids have been confined chiefly to observations made on homoiothermal vertebrates. Very little work has been done on the problem in the invertebrates.

Terroine and co-workers (1930) found that the phospholipids in various cold-blooded animals contain very low amounts of fatty acid, that the iodine number of the fatty acids is not constant, and that the iodine number of the phospholipid fatty acids is lower than that of the stored fat but higher than that in similar tissues of homoiothermes.

Timon-David (1930) found that the larvae of 24 insects contained 0.94–28 per cent fat and that linolenic and oleic acids were widely distributed.

Slifer (1932) found that the iodine number of the fatty acids in the eggs of seven species of grasshopper varies from 128–167. She found also that the temperature for complete fusion of the fatty acids depends on the environmental temperature: fats from eggs so laid as to hatch in spring had a melting point decidedly lower than fats from eggs so laid as to hatch in the summer.

Salisbury and Anderson (1939) found that the lipids in *Cysticercus fasciolaris* consist of phospholipids, cholesterol, cerebrosides, and a small amount of neutral fat; that the phospholipids make up about 30 per cent of the total lipids; and that the fatty acids contain saturated and unsaturated forms in the ratio of 1 : 1.

Wilber (1947) showed that starvation decreases the total lipid in the entire worm *Phascolosoma,* causes little change in the muscle lipids, and decreases markedly the fatty acid and phospholipid content of the coelomic fluid, but does not change the cholesterol content of the latter.

It is evident that information concerning lipid content and metabolism in the invertebrates is quite meager. A detailed study of the problem seems justified. The present report is one in a series dealing with the amounts and use of lipids in invertebrate tissues.

Material and Methods

Marine annelids were chosen for the investigation because they are obtainable readily and are easy to handle. The following species were studied: *Nereis pelagica, Amphitrite ornata, Arenicola marina, Phascolosoma gouldii, Lepidonotus squamatus, Glycera americana, Chaetopterus variopedatus.* All were procured in the living condition from the Supply Department of The Marine Biological Laboratory during the months of July and August, 1946.

Whole worms or individual tissues were prepared by grinding weighed amounts with sand in a mortar (Bloor, 1929). Lipids were then extracted with boiling alcohol. Some tissues were homogenized in a Waring-blendor instead of grinding with sand. The results from both methods are comparable.

Phospholipids were precipitated with acetone and magnesium chloride and estimated by the oxidation-titration method (Bloor, 1929). Fatty acid and cholesterol were estimated respectively by the oxidation-titration method and by the colorimeter using the acetic anhydride-sulfuric acid reagent (Bloor, 1928). This method measures the total fatty acids in the lipids.

Coelomic fluid was removed from some of the worms and the lipids were extracted and estimated in a similar manner.

From the results obtained, the lipocytic coefficients (cholesterol/fatty acid) and the ratio, cholesterol/phospholipid were calculated for the various tissues (Mayer and Schaeffer, 1913).

RESULTS

The results are summarized in Table I which shows that there is a wide variation in the absolute values of the various lipid constituents in the different species.

TABLE I

Table showing the amounts of various lipids (values given as per cent of wet weight) in marine annelids and the numerical ratios of the lipids one to another. Values are the mean of ten worms selected at random

Species	Phospholipids	Cholesterol	Fatty acid	$\frac{\text{Chol.}}{\text{F.A.}}$	$\frac{\text{Ch.}}{\text{Ph.}}$	Total lipid
Nereis pelagica	0.73	0.35	1.82	0.19	0.48	2.17
Glycera americana	0.82	0.50	2.25	0.22	0.61	2.75
Amphitrite ornata	0.83	0.55	2.60	0.21	0.66	3.15
Arenicola marina	0.35	0.29	0.93	0.31	0.84	1.22
*Coelomic fluid**	155	55	877	0.06	0.35	932
Chaetopterus variopedatus	0.33	0.16	0.62	0.26	0.48	0.73
Lepidonotus squamatus	0.23	0.40	3.28	0.12	2.0	3.68

* Values in mg./100 cc. of coelomic fluid.

The table shows also that tissues with a high content of phospholipid have a high content of cholesterol. A similar relationship is apparent between cholesterol and fatty acid. If the values for fatty acid are plotted against the values for cholesterol, the points are distributed along a straight line.

DISCUSSION

The results indicate that although there is a wide variation in the absolute values of phospholipid, fatty acid, and cholesterol in the different annelids, there is an apparently consistent ratio of these lipids one to another.

The phospholipids of the annelids studied make up about 37 per cent of the total lipid. This value is close to that obtained (30 per cent) by Salisbury and Anderson (1939) in *Cysticercus fasciolaris*.

It is known that the total lipid in the insect *Sphanarium purpurascens* consists mainly of free fatty acids (Giral, 1946). In the present investigation there is evidence of a predominance of fatty acid over cholesterol and phospholipid in annelid tissue.

The relationship between cholesterol and phospholipids is interesting. The worms with large amounts of cholesterol have a correspondingly large amount of phospholipid. A similar relationship obtains between cholesterol and fatty acid. This may indicate that in the annelids, as in the vertebrates, the ratio of lipids one to another is characteristic of the organ of an animal in a given species (Mayer and Schaeffer, 1913).

The results in general indicate that in the marine annelids, cholesterol is always associated with phospholipids and is in constant relation to them. Cholesterol is, therefore, probably a normal protoplasmic constituent in the annelids as in the vertebrates (Bloor, 1943).

Whether these relationships are constant under different environmental conditions (changes in temperature, salt concentration, or season) is not known at present.

SUMMARY

1. The amount of cholesterol, fatty acid, and phospholipid in various marine annelids was estimated.

2. It was found that worms with a high content of cholesterol have also large amounts of phospholipid and fatty acid.

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THE PROBLEM OF DIFFERENCES OF OFFSPRING IN RECIPROCAL CROSSES OF DROSOPHILA[1]

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In general, reciprocal crosses between genetically different groups of organisms yield identical hybrids. In some cases, however, the offspring from reciprocal crosses are different from one another in spite of like nuclear constitution. Such cases are of particular interest, since they bear on the problem of the role of extranuclear factors in development and heredity.

In *Drosophila melanogaster* a few examples have been described in which the developmental fate of a zygote depends in part on the genetic constitution of the mother. It seemed possible to obtain further data. A series of reciprocal crosses was performed and the expression of a sensitive indicator phenotype, caused by the mutant "Dichaete," was determined. Several of these crosses seemed to give evidence of a maternal effect. However, further inquiry showed that the significant differences between the outcome of reciprocal crosses were at least partly due to inhomogeneity within each cross, and that no conclusion as to the existence or non-existence of maternal effects was justified. The data and their analysis presented here serve to emphasize the need for detailed statistical study of the range of variability within, as well as between, sets of data under comparison.

MATERIALS AND BREEDING METHODS

The mutant phenotype "Dichaete" (*D*, 3-40.4-41 ±) is a very sensitive indicator of external and internal conditions prevalent during the development of *Drosophila melanogaster* (Sturtevant, 1918; Plunkett, 1926). A quantitative measure of such conditions may be obtained by counting the number of dorsocentral bristles on the thorax of Dichaete flies. Normal flies usually have four dorsocentrals while Dichaete flies may have from zero to four.

Plunkett has already given some data showing that the mean number of dorsocentrals is not significantly different in the Dichaete offspring from reciprocal crosses between Dichaete and normal flies. The procedure followed in the present study consisted in reciprocal crossing of Dichaete and non-Dichaete flies, the latter of which contained some other mutant genotype. In other words, the expression of Dichaete was measured in populations which not only differed in the maternal or paternal origin of the Dichaete genotype but also in the origin of the egg from a mother either free or in possession of some other mutant genotype. The mutants used in combination with Dichaete were vestigial (2-67.0) Minute (2) (2-12.9 ±),

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[1] The author wishes to express his appreciation to Dr. Curt Stern for his helpful advice and guidance throughout this investigation. To Dr. D. R. Charles the author wishes to acknowledge in particular his indebtedness for aid in the mathematical treatment of the data throughout.

Minute (3) *w* (3-79.7), Minute 4 (4), and polychaetoid (3-39 ± 4). These mutants hereafter will be referred to as *M*-2, *M*-3, *M*-4, *pyd, vg* and *D* (Dichaete). The choice of mutants used was based on the following considerations: (1) *vg*. Hersh and Ward (1932) have reported that *vg*/+ heterozygotes possess slightly different wing dimensions, dependent on the direction of the parental cross, vestigial by normal. It was thought that the maternal effect indicated by these findings might express itself also in a modification of the bristle reaction in *D* flies. (2) Minutes. The effect of a Minute constitution consists not only in the morphological modification of bristle size but also in a striking prolongation of larval growth (Dunn and Mossige, 1937). The physiological mechanism underlying this developmental prolongation is unknown, but it seemed worthwhile to test whether egg cells derived from Minute females differed from egg cells derived from non-Minute females in their effect on the Dichaete reaction. (3) *pyd*. The use of this mutant was based on the supposition that its action toward increase of bristles may possibly result in a difference of egg cytoplasm from *pyd* as opposed to normal females.

The initial problem was to obtain seven stocks which were highly alike except for the mutants to be tested (*D, M*-2, *M*-3, *M*-4, *pyd,* and *vg*). The method was to replace whole chromosome "marked" with dominant mutant genes by homologous chromosomes of the isogenic wild type stock "Canton-S." A laboratory stock with dominant marked genes in the second and third autosome, of the genotype +/+ ; *Cy/Pm; Sb-C/H* * was crossed with each stock of *D, M*-2, *M*-3, *M*-4, *pyd,* and *vg* and their progeny outbred for three generations with Canton-S stock. In case of crosses involving *D* or the Minutes, when all marked chromosomes, as well as the *X* and *Y*, had been replaced by homologous Canton-S chromosomes, the mutant female of the resultant stock was chosen as one parent of each subsequent cross with Canton-S males. This was done in order to produce as much homogeneity as possible in the mutant chromosomes by allowing crossing-over to occur. The crosses described above were maintained for fourteen generations; subsequent flies were maintained by mass matings with all female parents of such mass matings selected from the same bottle. The Canton-S flies referred to were reproduced in each generation by pair matings from a single bottle.

A slightly different breeding method was used with *vg* and with *pyd,* because the former is a recessive and the latter a very weak dominant; it was necessary to inbreed flies from the fourth generation in order to obtain the *vg* or *pyd* phenotype (fully expressed in homozygous condition only). Thereafter, the *vg* and *pyd* flies were alternatingly outbred to Canton-S and inbred until the fourteenth generation was reached and the *vg/vg* and *pyd/pyd* flies were used in the experimental crosses.

The experimental flies were obtained from reciprocal crosses between the approximately isogenic stocks of Dichaete and of *vg, pyd, M*-2, *M*-3, and *M*-4. Reciprocal crosses were also made between Dichaete and non-Dichaete siblings. Altogether, twenty crosses involving 456 cultures were made.

All crosses were maintained in a seven-shelved incubator ventilated by a forced draft fan. The temperature was maintained between 24.5 and 25.0 degrees Centigrade throughout the whole experiment. Crosses were indiscriminately mixed on the shelves, thus insuring that no one group of bottles occupied a "favored" position.

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* Symbols used here refer to mutant genes as follows: *C* = crossing-over suppressor, *Cy* = curly wings, *H* = lack of certain hairs, *Pm* = plum eye color, *Sb* = stubble bristles. For further details see Bridges and Brehme (1944).

Two culture methods were used. (1) In the "bottle" method, virgin females were collected over a two-day interval and each was put with a male in a 20 × 90 mm. shell vial containing a piece of agar-molasses substrate (unyeasted) to supply moisture. At the end of the two days, the pairs of flies were transferred to ordinary half-pint milk bottles with the usual cornmeal-molasses food (unyeasted). After an additional three days the parents were shaken out and the bottles returned to the incubator. (2) The "egg-count" method differs in a few respects from the "bottle" method. Virgin females were collected as before and pair-mated in 20 × 90 mm. shell vials for two days. The pairs were then removed and put into half-pint milk bottles containing egg-laying dishes. These were rectangular metal dishes, 60 × 30 mm. long and wide, and 10 mm. deep, filled with food. The same substrate was used as above. Egg counts were then made. When 120 eggs had been deposited on the surface of the substrate, 12–36 hours later, it was transferred to a $4\frac{1}{2}$-inch finger bowl with yeasted food medium. The bowl was then covered with cotton gauze and another finger bowl and placed in the incubator. When all larvae had pupated they were transferred to bottles and remained there until eclosion.

EFFECT OF CULTURE METHOD ON POPULATION DENSITY

Considerable variability in size of population per bottle and in average population per cross was found with either culture method. The area of substrate per larva (as judged by flies surviving to eclosion) also differed greatly. Table I contains relevant data.

TABLE I

Effect of culture method on population density

	Egg-count method. Area of agar surface 9710 mm.^{2}		Bottle method. Area of agar surface 2633 mm.^{2}	
	Total population surviving	Area per surviving larva	Total population surviving	Area per surviving larva
Least densely populated bottle	33	294	75	35
Least densely populated cross (average)	69	141	100	26
Average of all crosses	82	118	115	23
Most densely populated cross (average)	95	102	130	20
Most densely populated culture	123	79	189	14

The two methods offered very different conditions of existence to the larvae. If these conditions had any significant effect upon bristle number, identical crosses cultured by the two methods would not be expected to yield comparable results.

Analytical Methods

The Dichaete offspring of the experimental crosses were classified by sex and number of dorsocentral bristles. No distinction was drawn between M and M^+ flies. As an index, the mean number of posterior dorsocentral bristles per hemithorax was computed separately for each sex in each bottle. In computing the index, one-half the number of one-bristle flies were added to the total number of two-, three-, and four-bristle flies, and this resulting sum divided by the number of flies of that sex in that bottle. This method of calculating posterior dorsocentral bristle frequency is justified by the data of Plunkett (1926) and Walker (1941). According to these workers, the effect of Dichaete is to remove the anterior dorsocentral bristles first and the posterior dorsocentral bristles secondarily, if the effect is strong enough. This means that if two bristles remain, they are practically always the posterior dorsocentrals, and if one bristle is present it is with about equal frequency the right or left posterior dorsocentral. As an overall measure of bristle frequency (in one sex) in a particular cross, a mean bottle index ($\bar{P}_{\text{cross}}$) was calculated in the same manner as for a single bottle. A summary of cross indices is shown in Table II.

Table II

Bristle frequencies (as measured by presence of posterior dorsocentrals per hemithorax; wild-type = 1) for Dichaete progeny of the experimental crosses. "A" and "B" refer to reciprocal crosses, the former denotes the cross in which the female *parent is Dichaete and the latter denotes the cross in which the* male *parent is Dichaete.*

Experimental cross number	Mutant used to test for "maternal" effect	Female progeny		Male progeny	
		A	B	A	B
		Mutant father	Mutant mother	Mutant father	Mutant mother
		Cross index		Cross index	
1*A* and 1*B*	*vg* (bottle method)	0.704	0.752	0.529	0.635
**2*A* and 2*B*	*vg* (egg-count method)	0.774	0.710	0.681	0.403
3*A* and 3*B*	*M*-4 (bottle method)	0.432	0.363	0.289	0.180
4*A* and 4*B*	*M*-3 (bottle method)	0.585	0.601	0.423	0.445
**5*A* and 5*B*	*M*-3 (egg-count method)	0.824	0.903	0.625	0.605
6*A* and 6*B*	*M*-2 (bottle method)	0.669	0.529	0.500	0.425
7*A* and 7*B*	*pyd* (bottle method)	0.697	0.596	0.660	0.445
8*A* and 8*B*,	Dichaete* (egg-count method)	0.855	0.853	0.783	0.718
9*A* and 9*B*,		0.779	0.793	0.599	0.523
10*A* and 10*B*		0.843	0.891	0.671	0.709

* These experiments were carried out within the Dichaete stock itself; *D*/*C* females and males were crossed reciprocally with *C*/*C* males and females.

** These crosses done by the "bottle" method and repeated later by the "egg-count" method.

Whether the indices (for the same sex) of the reciprocal crosses were or were not significantly different was determined in three steps:

(1) for each sex ($\bar{P}_{\text{females}}$ and $\bar{P}_{\text{males}}$), a joint mean of the two reciprocal crosses was calculated from the combined data of the two crosses in the same manner as for a single bottle.

(2) the bottles above and below the joint means, sexes considered separately, were entered in a four-fold table.

(3) this table was tested for homogeneity by the usual χ^2 method. The relevant data for all crosses are summarized in Table III which shows the probability that such a distribution above and below the joint index could be a chance occurrence.

TABLE III

χ^2, n, *values for distribution of bottles whose indices fall above and below the joint mean* ($\bar{P}_{\text{females}}$ *and* $\bar{P}_{\text{males}}$) *for each reciprocal cross.*

Crosses compared		Female progeny		Male progeny		Combined progeny	
No.	♀ × ♂	$(n=1)$ χ^2	P	$(n=1)$ χ^2	P	$(n=2)$ χ^2	P
1*A* *1*B*	*D/C* × *vg/vg* *vg/vg* × *D/C*	5.127	0.025	4.282	0.042	9.409	0.010
*2*A* 2*B*	*D/C* × *vg/vg* *vg/vg* × *D/C*	5.432	0.020	24.966	0.003	30.398	<0.010
*3*A* 3*B*	*D/C* × *M-4/C* *M-4/C* × *D/C*	2.785	0.100	2.115	0.160	4.900	0.089
4*A* *4*B*	*D/C* × *M-3/C* *M-3/C* × *D/C*	2.131	0.154	1.054	0.306	3.185	0.204
†5*A* **5*B*	*D/C* × *M-3/C* *M-3/C* × *D/C*	3.343	0.072	0.016	0.900	3.359	0.190
*6*A* 6*B*	*D/C* × *M-2/C* *M-2/C* × *D/C*	9.185	0.007	6.417	0.011	15.602	<0.010
*7*A* 7*B*	*D/C* × *pyd/pyd* *pyd/pyd* × *D/C*	13.616	<0.010	18.169	<0.010	31.885	<0.010
*8*A* 8*B*	*D/C* × *C/C* *C/C* × *D/C*	0.744	0.407	3.702	0.056	4.446	0.115
†9*A* **9*B*	*D/C* × *C/C* *C/C* × *D/C*	0.628	0.444	1.563	0.219	2.191	0.392
10*A* *10*B*	*D/C* × *C/C* *C/C* × *D/C*	3.478	0.067	0.965	0.332	4.443	0.117

* Cross giving higher index for progeny of both sexes.

** Cross giving higher index for female progeny.

† Cross giving higher index for male progeny.

Of the mutants tested, three—*pyd, vg,* and *M*-2—have significantly different indices in reciprocal crosses; two, *M*-3 and *M*-4, do not, nor does Dichaete itself.

One totally unexpected feature of these results is that the difference between reciprocal crosses involving *vg* was of opposite sign in the two trials, but significant in each case. In crosses 1*A*(*D/C*♀ × *vg/vg*♂) and 1*B*(*vg/vg*♀ × *D/C*♂) both male and female offspring had higher indices where the vestigial parent was the *mother;* in crosses 2*A*(*D/C*♀ × *vg/vg*♂) and 2*B*(*vg/vg*♀ × *D/C*♂) each sex showed a larger index where the vestigial parent was the *father.* Among many possible explanations, three seem most worthy of consideration. (1) Inefficient statistical methods may have been used. It may be recalled here that the general purpose of statistical tests of the reality of observed differences is to predict whether similar differences would be expected in repetitions under comparable conditions. Predictions are based here on the variation of bottle indices *within* an experiment. In the present case the statistical test of either *one* experiment of a pair leads to the expectation that the other experiment should yield a difference of the same sign, if it has been performed similarly. The other, however, gives opposite results. (2) There may have been a difference in procedure between the two experiments of a sort which, in general, reverses the direction of difference between reciprocal crosses involving *vg* and Dichaete. In fact crosses 1*A*(*D/C*♀ × *vg/vg*♂) and 1*B*(*vg/vg*♀ × *D/C*♂) were raised by the bottle method and had average population densities of 4.7 and 4.4 larvae per cm.^{2} (as judged by the number surviving to eclosion); crosses 2*A*(*D/C*♀ × *vg/vg*♂) and 2*B*(*vg/vg*♀ × *D/C*♂), raised by the egg-count method, had densities of 0.85 and 0.91 larvae per cm^2. (3) There may have been a difference in material between the two experiments. In fact the vestigial parents used in the two trials were not of undoubtedly identical genotype. In maintaining the *vg* and Canton stocks, a contamination was noticed in both of them between the first and last *vg* experiments. The Canton stock was re-obtained from a laboratory stock which had come originally from the same stock as the first Canton stock but had been inbred by mass matings instead of pair matings. A stock of vestigial was obtained from the same source as before and was made approximately isogenic by the same procedure as before. On the whole, then, explanation (3), difference in stock, seems relatively improbable.

Explanation (1), inefficient statistical method, also may reasonably be excluded: the method is standard and its prediction about repetitions is upheld in the one cross which was repeated without change in culture method. Three sets of reciprocal crosses were made between Dichaete and non-Dichaete siblings. In each set of reciprocal crosses the indices for both sexes were found not to be significantly different. The prediction from the statistical tests is also fulfilled in one case where the culture method was changed, that is, crosses 4*A*(*D/C*♀ × *M*-3/*C*♂) and 4*B*(*M*-3/*C*♀ × *D/C*♂) were done by the bottle method and their indices (for both sexes) were found not to be significantly different. Later, this same pair of reciprocal crosses was repeated (cross 5*A*: *D/C*♀ × *M*-3/*C*♂ and cross 5*B*: *M*-3/*C*♀ × *D/C*♂) and the progeny raised by the egg-count method. Here, as before when the bottle method was used, neither sex showed indices significantly different from those of their reciprocal crosses.

Thus explanation (2), effect of culture method, seems to be the only one of the three leading possibilities which cannot be reasonably excluded. That it should be *expected* to bring about a reversal of the difference between reciprocal crosses is not obvious from the literature on bristle phenotypes. That it may actually have done so can scarcely be decided without some study of the relation between bristle index and the factors which change with varying culture conditions. It is with this problem that the following section is concerned.

FACTORS AFFECTING THE BRISTLE INDEX

Three factors affecting the bristle index will be considered.

(1) *Differences within crosses.* Whatever the factors may be which affect the bristle frequency in the present material, they do not operate identically even through a group of bottles prepared at the same time, containing progeny from parental pairs of the same genotype, and all incubated within 0.5 degrees Centigrade of the same temperature. This fact was established by making χ^2 tests for homogeneity for the female indices within each of the twenty experimental crosses. The results of these tests are listed in Table IV. Altogether, in twelve of the twenty crosses, significant differences among bottle indices for females were found between duplicate bottles of the same cross.

TABLE IV

χ^2 *treatment of the comparison of bottle and group indices of the female progeny within crosses 1A–10B*

Cross		χ^2	n	P
No.	♀ × ♂			
1*A*	*D/C* × *vg/vg*	94.179	34	<0.010
1*B*	*vg/vg* × *D/C*	64.463	16	<0.010
2*A*	*D/C* × *vg/vg*	55.793	29	<0.010
2*B*	*vg/vg* × *D/C*	105.025	35	<0.010
3*A*	*D/C* × *M-4/C*	47.372	30	0.024
3*B*	*M-4/C* × *D/C*	12.815	17	0.747
4*A*	*D/C* × *M-3/C*	104.200	37	<0.010
4*B*	*M-3/C* × *D/C*	108.071	40	<0.010
5*A*	*D/C* × *M-3/C*	18.895	18	0.407
5*B*	*M-3/C* × *D/C*	9.082	14	0.823
6*A*	*D/C* × *M-2/C*	132.111	29	<0.010
6*B*	*M-2/C* × *D/C*	143.243	45	<0.010
7*A*	*D/C* × *pyd/pyd*	72.103	31	<0.010
7*B*	*pyd/pyd* × *D/C*	93.845	45	<0.010
8*A*	*D/C* × *C/C*	25.115	19	0.163
8*B*	*C/C* × *D/C*	18.893	17	0.339
9*A*	*D/C* × *C/C*	42.234	39	0.674
9*B*	*C/C* × *D/C*	71.068	36	0.013
10*A*	*D/C* × *C/C*	26.987	39	0.162
10*B*	*C/C* × *D/C*	72.094	41	<0.010

(2) *Differences related to eclosion order.* In addition to the unexplained differences among bottles treated as similarly as possible, consistent differences in bristle index are also found within individual bottles. In each of the crosses raised by the bottle method, the first flies to eclose showed a higher mean bristle

index than the flies included in the second count, two days later. And these in turn had a higher mean index than flies included in the third count which was four days after the first. That is, in general, the bristle index decreased with eclosion order.

The underlying factor or factors which relate the bristle number of an individual fly to its eclosion order are unknown. They may include, among others, amount of moisture of food, presence of metabolic wastes of larvae, and amount of yeast available during all or part of the life of a larva. The yeast growth of a particular culture presumably varies partly with the number of larvae which have previously been feeding upon it, and perhaps partly with elapsed time, independently of larval population.

(3) *Difference related to area per eclosed larva.* It was found in all but one out of sixteen cases that bristle index was positively correlated with substrate area per eclosed larva. The positive correlation coefficients were found to vary from 0.479 in the male progeny of cross 2*A*(*D*/*C*♀ × *vg*/*vg*♂) to 0.049 in the male progeny of cross 2*B*(*vg*/*vg*♀ × *D*/*C*♂) (Table V). The excessive variability of indices within crosses, and the influence on size of index of eclosion order, makes difficult an evaluation of the correlation between index and substrate area. A clarification should be based on data containing counts of daily hatches and not only, as in the data used, on complete hatches.

TABLE V

Correlation coefficients between bristle index and substrate area

Cultured by	Cross	Female progeny	Male progeny
		r	*r*
Bottle method	1*A* (*D*/*C* × *vg*/*vg*)	0.303	0.160
Bottle method	1*B* (*vg*/*vg* × *D*/*C*)	0.471*	0.078*
Egg-count method	2*A* (*D*/*C* × *vg*/*vg*)	0.099*	0.479*
Egg-count method	2*B* (*vg*/*vg* × *D*/*C*)	0.085	0.049
Bottle method	4*A* (*D*/*C* × *M*-3/*C*)	0.092	0.084
Bottle method	4*B* (*M*-3/*C* × *D*/*C*)	0.309	0.321
Egg-count method	5*A* (*D*/*C* × *M*-3/*C*)	0.328	0.257
Egg-count method	5*B* (*M*-3/*C* × *D*/*C*)	−0.037	0.228

* Signifies cross with significantly different mean bristle index in reciprocal crosses.

DISCUSSION

The original purpose of this investigation was to test for "maternal effect," *i.e.*, phenotypic effects expressed exclusively through or by the maternal parent, in the offspring of reciprocally crossed isogenic stocks. No significant differences were found between reciprocal crosses involving *M*-3, *M*-4, nor within Dichaete itself, but significant differences between reciprocal crosses involving *M*-2, *pyd*, and *vg* were found (as judged by mean posterior dorsocentral bristle index). However, two groups of data showed large and unexplained variation. These were the significant differences in mean bristle index of cultures *within* crosses, and the reversal in sign of the significant differences between crosses 1*A*(*D*/*C*♀ × *vg*/*vg*♂) and 1*B*(*vg*/*vg*♀ × *D*/*C*♂), and crosses 2*A*(*D*/*C*♀ × *vg*/*vg*♂) and

$2B(vg/vg\,♀ \times D/C♂)$. In regard to the former, these significant differences arose among progeny of parental pairs of identical age and genotype. They arose in spite of efforts to produce, as nearly as possible, identical treatment of all flies. It must be concluded that these efforts were not sufficiently successful in making the environments of different sets of progeny homogeneous. Such factors as the condition of the substrate, the availability of yeast, and possibly the presence of bacteria and fungi in the fermenting medium were probably of influence on the bristle indices. With regard to the reversal in difference between bristle indices in the two sets of *vg*-Dichaete crosses, the unexplained variability just discussed may be the only cause of the opposing results. In addition, it should be pointed out that different culture methods were employed in the *vg*/Dichaete experiments. During larval life the closure of the experimental dishes in the "egg-count" method by means of cheesecloth and fingerbowls is considerably less tight than in the bottle method. It allows for more loss of substrate moisture and permits perhaps greater inoculation with bacteria and fungi. Furthermore, the greater area of substrate per larva in the "egg-count," as compared to the bottle method, presents a difference in environment, in degree if not in kind, to the developing larvae. The availability of yeast and the abundance of competitive organisms may be altered, as part of this environment, and in turn may influence the production of posterior dorsocentral bristles. Whether the effects of these two different environments is of such nature as to cause a significant reversal of mean bristle index between these two sets of reciprocal crosses is not conclusively shown in these data. The fact, however, that they may influence mean bristle index in experimental flies should not be ignored.

In the interest of more exact and uniform conditions, certain refinements of technique in future experiments may be mentioned here. It is well known that too few as well as too many larvae do not produce healthy culture conditions. Therefore, the optimal area per substrate per larva should be determined. In view of the fact that great variations in egg hatching are sometimes encountered, it is suggested that, where possible, counted larvae are chosen for experimental material rather than eggs. The substrate itself varies somewhat from culture to culture in the amount of moisture present and acidity (Bridges and Darby, 1933). Buffered substrate and controlled moisture conditions might aid in establishing more uniform culture conditions.

Differences between reciprocal crosses somewhat similar to those encountered in this work have also been described for *vg* by Hersh and Ward (1932), and Child (1939). No data were presented dealing with differences in wing size related to eclosion order, or to amount of substrate per larva, nor have tests of homogeneity been reported. The present work points to the necessity for extremely well controlled culture conditions and a searching statistical analysis, before conclusions concerning "maternal effect" can be accepted. In view of the residual, uncontrolled variability, the data obtained in this work are inconclusive as to the presence or absence of a "maternal effect."

SUMMARY

1. To test for the existence of maternal effects in *Drosophila melanogaster*, approximately isogenic stocks of Dichaete, *pyd*, *vg*, *M*-3, *M*-4 were prepared.

Reciprocal crosses were made between each stock and Dichaete, and between Dichaete and non-Dichaete siblings. The results, as indicated by posterior dorsocentral "bristle indices" (*i.e.*, average number of dorsocentral bristles per hemithorax), of the Dichaete progeny show that no significant differences arose within six pairs of reciprocal crosses. The reciprocal crosses of four other pairs of experiments yielded significant differences in bristle indices. Two of these pairs were alike in type of crosses, but different in type of culture method. The sign of the differences in bristle indices was reversed in these two sets of experiments, the progeny with the higher indices coming from Dichaete mothers in one case, and from Dichaete fathers in the other.

2. Tests of homogeneity show that within crosses, all bottles of which have had identical treatment, differences in mean posterior dorsocentral bristle frequency occur which are greater than those to be expected on the basis of simple sampling errors.

3. The size of the bristle index of an individual fly depends partly upon its eclosion order, with those flies that eclose earliest having the highest bristle index.

4. The size of the bristle index depends partly on area of substrate available to the individual, although this dependence may be weak in many cases.

5. The lack of homogeneity in indices within crosses, and the reversal in direction of "maternal effect" in the pairs of crosses grown under two different culture conditions, suggest that external conditions during the development of the flies must be made more constant than heretofore, before conclusions as to the presence or absence of a "maternal effect" can be drawn.

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BINARY ANTERIOR OCELLI IN ANTS

NEAL A. WEBER

In a paper by this name in the *Biological Bulletin* William Morton Wheeler (1936) published an account of binary anterior ocelli which was believed to be the first record of this condition in adult insects. He was led into a search of the literature by finding a doubling of the anterior ocellus in 15 out of more than 4,000 of a colony of anomalous ants (*Cephalotes atratus quadridens* DeGeer), which he called gynandromorphs. This colony was one which I collected in Trinidad, British West Indies. The discovery led him to examine other ants, an examination which revealed a comparable condition in a soldier of the Indomalayan ant, *Pheidologeton diversus laotina* Santschi, and in more than 60 per cent of a series of 300 soldiers from 25 colonies of the neotropical leaf-cutting ant, *Atta cephalotes* L.

The anterior ocellus of the insect eye has long been known to have a double innervation, while the lateral ocelli have a single innervation. In the ontogeny of insects there are also two primordia for the anterior ocellus itself, one for each of the lateral ocelli.

It is the purpose of this note to give credit to a much earlier discoverer of the binary anterior ocellar condition in ants and to add several additional records.

H. W. Bates in *"The Naturalist on the River Amazons,"* of which the first edition was published in 1863, while writing of the large leaf-cutting ants or Saüba at Pará, Brazil, says:[1]

"The third order of workers is the most curious of all. If the top of a small, fresh hillock, one in which the thatching process is going on, be taken off, a broad cylindrical shaft is disclosed. . . . If this be probed with a stick . . . a small number of colossal fellows (Fig. 3) will slowly begin to make their way up the smooth sides of the mine. Their heads are of the same size as those of the class Fig. 2; but the front is clothed with hairs, instead of being polished, and they have in the middle of the forehead a twin ocellus, or simple eye, of quite different structure from the ordinary compound eyes, on the sides of the head. This frontal eye is totally wanting in the other workers, and is not known in any other kind of ant."

A new record which is here figured (Fig. 1) is that of another fungus-grower, *Acromyrmex* (A.) *coronatus* Fab. In this genus, however, there is no soldier caste, although the workers are otherwise as polymorphic, and the large workers do not normally have ocelli. Three large workers from Bolivia (Rosario: L. Rocagua, W. M. Mann, collector) show the condition as represented. The frontal area, above the convex posterior margin of the clypeus and between the bases of the antennal scapes, is shown. The ants are normally rugose here and this condition is indicated by short irregular lines. The dotted lines constituting the margin of each figure represent the frontal carinae which are expanded anteriorly as frontal lobes covering the insertions of the antennae. In the middle of each frontal area posteriorly lie the paired ocelli. Each pair is in the position of the normal single anterior ocellus of the functional female caste of the species. In the female of *A. coronatus globoculis* Forel, for example, the lateral ocelli lie immediately outside the frontal carinae in the position indicated in the figures by a pair of short, converging, dotted lines forming the most posterior portion of the drawings.

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[1] His description and figures (Everyman's Libr. ed., 1930, pp. 10–16) of the large, shiny-headed soldier with distinct ocelli fits *Atta cephalotes* L. and this is the species he names (as *Oecodoma cephalotes*). He, however, states that the male is not much more than half the size of the female and this is characteristic of *Atta sexdens* L., the common Brazilian species. It is possible that most of his extensive account, which is not quoted, refers to *sexdens* and the condition of double ocelli refers to *cephalotes*. When he returned to England he may have examined the latter in collections and then discovered the ocelli, or he may have seen the condition in life along the upper Amazon where *cephalotes* and *laevigata* occur.

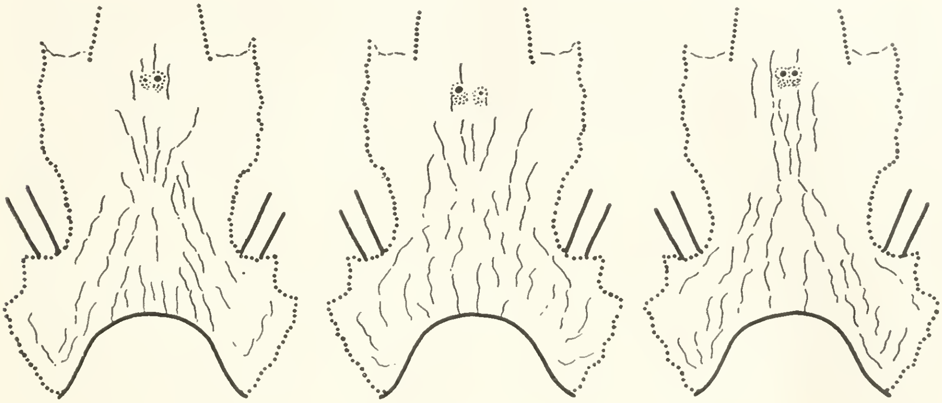

Figure 1

Other species of fungus-growers in my collection show binary anterior ocelli or other variations from the usual absence of ocelli.

Soldiers of *Atta cephalotes* from Peru show the condition described by Dr. Wheeler. The three mounted maxima soldiers of the type colony of *A. cephalotes isthmicola* Weber from Panama have large binary anterior ocelli and vestigial or no lateral ocelli. Three smaller soldiers of the same colony have each a minute anterior ocellus and no lateral ocelli.

Out of three soldiers of *Atta sexdens rubropilosa* Forel (det. Gonçalvez) from Rio de Janeiro, only the largest shows ocelli and these are a minute lateral pair. Three soldiers of *A. sexdens robusta* Borgmeier from Rio de Janeiro (Gonçalvez) show minute anterior and much larger lateral ocelli.

Large soldiers of *Atta laevigata* (F. Smith) from Venezuela, Brazil and Bolivia have a variable anterior ocellus which in some is smaller, in others larger, than the lateral ocelli. Several of the anterior are binary.

It may be pointed out that all of these records, now comprising four genera, are from the subfamily Myrmicinae which occupies a position about midway in the phylogeny of ants.

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AN ELECTRON MICROSCOPE STUDY OF PROTOZOAN FLAGELLA

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Introduction

While the magnifications obtained by the electron microscope have added greatly to the knowledge of the morphology of bacteria, the protozoa have been found to be too thick for easy penetration (unpublished work). However, it was believed that certain protozoan structures could be studied to advantage, and the following paper summarizes some results of electron microscope studies of flagella.

Flagella have been studied extensively with the light microscope. Vlk (1939) demonstrated several types among which was the ciliary type consisting of a shaft on which were located numerous small cilia, or very fine fibrils. Other biological fibers have been studied and are being investigated by means of the electron microscope. Jakus (1945) studied the morphology of trichocysts of *paramecia* elucidating the "ultra-structure" of the shaft. The shaft material is made of fibers which show long spacings typical of contractile fibers. These spacings, which probably represent the fundamental molecular arrangement in the fiber, differ slightly in length from the spacings in myosin and collagen. The connective tissue fiber, collagen, has been studied by Schmitt, Hall, and Jakus (1942) using the electron microscope. They were able to measure directly the long spacings which had previously been studied by Clark, Parker, Schaad, and Warren using X-ray diffraction techniques (1935), and had been remeasured by Bear (1942, 1944). Hall, Jakus, and Schmitt (1945) demonstrated several distinct long spacings in muscle fibers.

Fibers have been observed by electron micrograph in the tails of sperm by Baylor, Nalbandov, and Clark (1943) using bull sperm, and by Harvey and Anderson (1943) using the sperm of the sea urchin. The axial filament of the sperm tail in unfixed preparations separated into about ten to fifteen fibrils either in the region of the naked filament or in the region of a break in the protoplasmic sheath. The flagella of bacteria have been studied by Mudd, Polvitsky, and Anderson (1942) using the electron microscope.

By all odds the outstanding investigation on flagella, both in structure and function, is that of Harley P. Brown (1945). This Ph.D. thesis gives an exhaustive historical background as well as an account of the author's extensive experiments ranging from electron micrographs to underwater swimming. This paper unfortunately did not come to the attention of the present authors until a considerable amount of our work had been completed. Actually, there is very little to be added to the work of Brown. But it seems of value to record our independent observations, most of which are on quite different organisms. Our electron micrographs in part confirm the data of Brown, but those included in this paper primarily present new information on flagella not disclosed in his electron micrographs.

Brown studied the following organisms: *Astasia kelebsii* Lemmermann, *Euglena gracilis* Klebs, *Ochromonas variabilis* Meyer, and *Chilomonas paramecium* Ehrenberg. The micrographs show that the flagella are of approximately uniform diameter throughout their entire length; that each flagellum consists of a denser axial core (axoneme) and a less dense sheath surrounding the core; that in the flagella of *Euglena* and *Astasia* the axial core appears to consist of two fibers of equal size; that the sheath appears to contain or consist of a coiled fiber in a helix encircling the axial core; that flagella of *Euglena* and *Astasia* bear along one side a single row of delicate filaments, designated flimmer, while the long flagellum of *Ochromonas* bears similar flimmer along both sides, and that of *Chilomonas* bears none. The general conclusion of Brown is that the flagellum beats in spiral undulations, confirming the theory of Lowndes (1945).

Materials and Methods

The R.C.A. electron microscope, type B, was used in the following experiments. The microscope and its application to biology has been described in detail by Marton (1941), and by Zworykin, Marton, Ramberg, Hillier and Vance (1945). The specimen was placed directly on a collodion membrane supported by a standard wire mesh which served as the "slide." In the experiments which follow it was often desirable to examine the specimens at low magnifications so as to correlate the results with those of the light microscope. It was also desirable to view a wide field. The wide field, low magnification and greater depth of focus were obtained by placing the "slide" in the top of the specimen holder. The optical principles involved have been described by Burton, Barnes, and Rochow (1942). The technique of calibration described in that paper was employed to obtain the magnification figures used in this work.

Mixed cultures of protozoa were obtained from water from the Boneyard Creek in Urbana, Illinois. They were subcultured in chlorine-free water to which a grain of unpolished rice was added. Some pure cultures were attempted by subculturing from single organisms picked from dilute mixed cultures by fine pipette, but results of this procedure were unsatisfactory both as to photographs obtained and purity of culture. Saline suspensions of the gut contents of the cockroach *Peraplanita americana* were prepared in an attempt to photograph the flagella of the flagellates *Lophomanas blattarum* and *L. striata*. In a similar manner, suspensions of the gut contents of the termite *Reticulitermes flavipes* were prepared in order to observe the flagella of this rich flagellate fauna.

Specimens were prepared in the following ways:

1. A drop of culture was placed directly on the collodion membrane and allowed to dry in air. Some of these were washed in distilled water to remove excess debris, organisms, or salt.

2. A drop of the culture was placed directly on the collodion membrane, allowed to stand five to ten minutes in air, and then before complete drying had occurred, the screen was touched to a meniscus of distilled water. The surface tension of the meniscus drew the liquid from the screen leaving the biological material attached to the membrane.

3. Organisms were fixed in formalin directly on the screens, which were then washed in the above manner to remove the excess formalin.

4. Mixed cultures were fixed and stained with osmium tetroxide by adding 2 per cent OsO_4 in aqueous solution to the drop containing the organisms, allowing the organisms to sink, and then pipetting them to the screen.

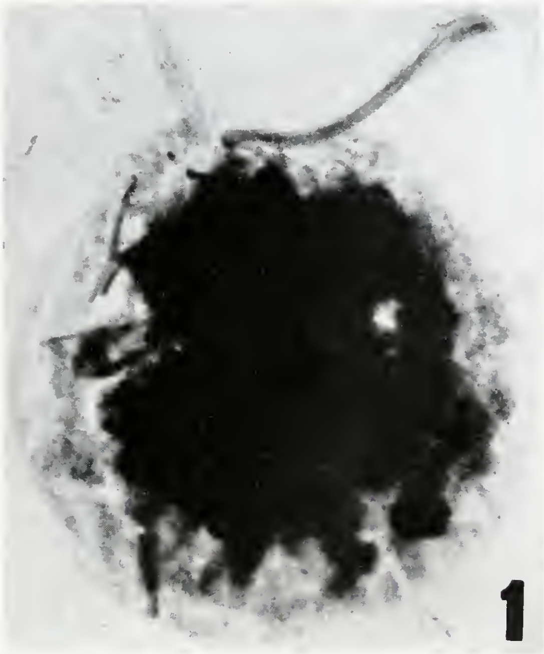
RESULTS

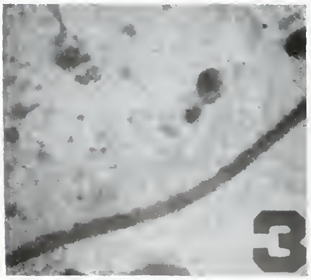
Most of the protozoa were too large and thick for penetration by the electron beam. Specimens of *Euglena* sp. allowed no penetration, either in the region of the cell body or through the flagellum itself. Preparations from the gut of both cockroach and termite contained sufficient extraneous matter to obscure the structural details of the locomotor organelles of the flagellates. However, flagellates from the fresh-water cultures, some of which were tentatively assigned to the genus *Monas*, did allow enough penetration after drying to permit the cell outline and granules within the cell to be observed. Since this favorable material possessed several different and distinct types of flagellar forms, it was used for most of the studies herein described.

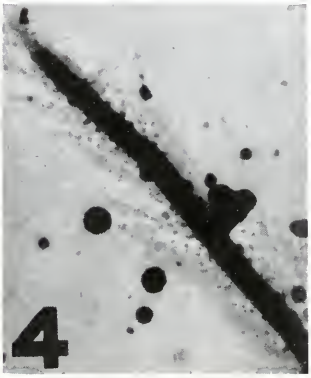
The general cell form of the organisms studied can be observed from the electron micrographs. Figure 1 shows a round cell body of *Monas* sp. which has undoubtedly flattened and shrunk during drying. The cell membrane is clearly seen, as is the protoplasmic mass which has become distorted during drying. Numerous granules can be seen within the cell, but their identification can only be surmised. The unidentified flagellate in Figure 2 shows an invagination at the apex behind which is a clear area, suggesting a cytostome and reservoir, or perhaps a contractile vacuole.

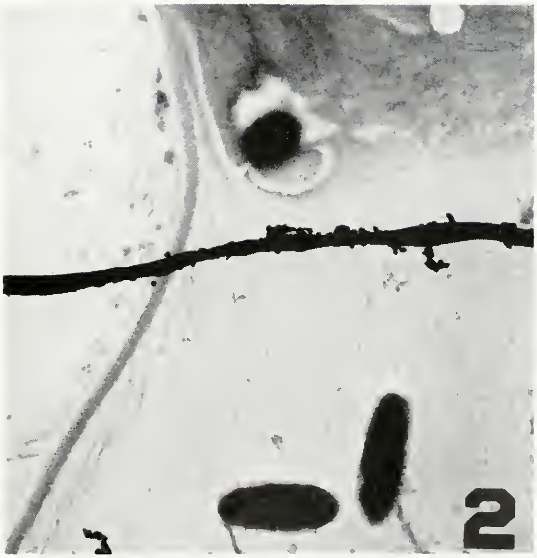
The fixation techniques employed in this work were not of a nature to demonstrate delicate cellular or flagellar components. Denaturation of protein appears to render it more opaque to the electron beam as does certainly the addition of a heavy metal. Formalin fixation caused adherence of foreign material to the cell body. The effects of osmium tetroxide can be seen in Figures 4, 6, 7, and 10. Much debris is always adsorbed and the cell body is completely impenetrable. The freezing-drying technique (Wykoff, 1946) is suggested as being probably the best to preserve the form of these animals for study with the electron microscope.

The flagella seen in the accompanying photographs are of two types, the ciliary flagellum and the fibrous flagellum. Vlk (1938) named the ciliary flagellum and found it present in many species of flagellates. He called the type without cilia the whip-flagellum, which may be the same as the type observed in this work and designated as the fibrous flagellum owing to the nature of its structure. The ciliary type seen in Figures 1 through 7 appears to consist of a shaft or tube, or mass of densely packed elongate fibers surrounded by radially placed ciliary structures (or flimmer). The exact nature of the central shaft cannot be determined from the present photographs. At the distal end of the flagellum shown in Figure 1, and in the region of the bend in Figure 5 long heavy fibers may be seen. The shaft may be a tube of these, or may have them embedded in the tube wall as a contractile element. In Figures 2 and 3 the shaft appears solid. In Figure 7 the central structure, which has been fixed with osmic acid, may possibly be interpreted as a hollow tube. It is probable that there are several different sub-types of the ciliary type of flagellum. In numerous cases one of the two flagella measured less than one micron in length. Flagella usually measured from 5 to 10 microns in length, and about 0.3 microns in width.









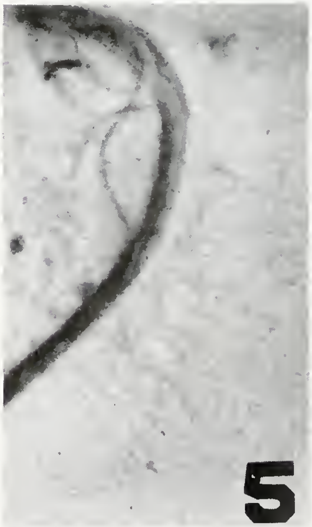


PLATE I

FIGURE 1. Electron micrograph of "*Monas* sp." showing cell body and attachment of two types of flagella (ca. 5,700 ×).

FIGURE 2. Organism (unidentified) showing single ciliary flagellum. Note shaft nodules and arrangement of cilia (ca. 4,700 ×).

FIGURE 3. Portion of ciliary flagellum showing nodules of shaft and cilia (ca. 6,600 ×).

FIGURE 4. Ciliary flagellum after osmic acid fixation (ca. 8,400 ×).

FIGURE 5. Ciliary flagellum with fibrous shaft (ca. 9,600 ×).

The point of attachment of the cilia on the flagella could not be determined by these photographs. Figures 2 and 3 show small nodules limited to one side of the shaft from which groups of cilia appear to take their origin. These are most dense in the proximal region. Brown ascribed this appearance to the helical fibrous sheath closely appressed to the axial core. Figures 1 and 5 show no such nodules although the cilia are clearly seen. In Figure 4 the form of the shaft is obscured by clumps of osmic acid which have also deposited between the individual cilia.

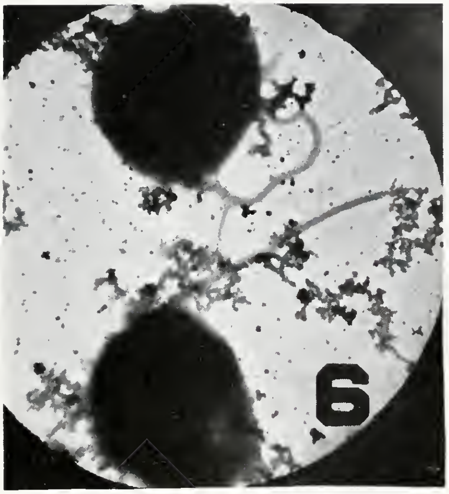
The fibrous flagella are best seen in Figures 8 and 9. They appear to be long fibers twisted spirally as the fibers of a rope. The rope-like quality is especially clear in Figure 9 where the tip of the flagellum has frayed. This structure was not observed in the microscope of Brown. Figure 8 shows the base of a flagellum which has broken away from the rest of the cell. These were not uncommon. The fibers appear to extend into the circular portion which is probably a part of the cell body. It would be interesting to observe isolated portions of the cell membrane for long fibers.

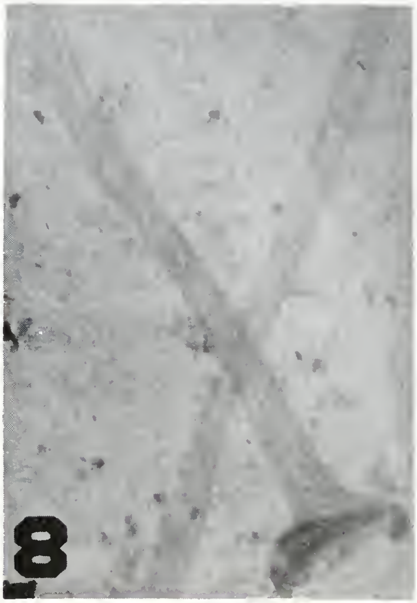
Long spacings were sought in the fibers. Enlargement of the fiber showed none. However, only very excellent photographs at high magnifications and at perfect focus can be used for these determinations. In osmic acid fixed rat tail collagen we were able to observe the long spacings reported by Schmitt, Hall and Jakus (1942). Hall, Jakus and Schmitt (1945) were able to demonstrate spacings in myosin only after treatment with either osmic acid or phosphotungstic acid. Figure 10 is a high magnification of the flagellar fibers treated with osmic acid. The granulation seen in the fibers is very regular, and paired rows of granules appear to lie together. This is the only indication of a periodicity which has been found in this work. It may be an artifact caused by precipitated osmic acid enmeshed in the fibrils. Very careful additional work should be carried out in this direction.

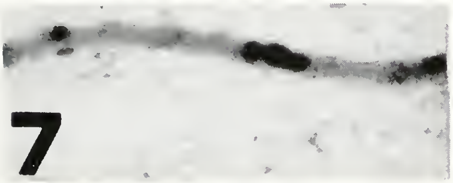
DISCUSSION

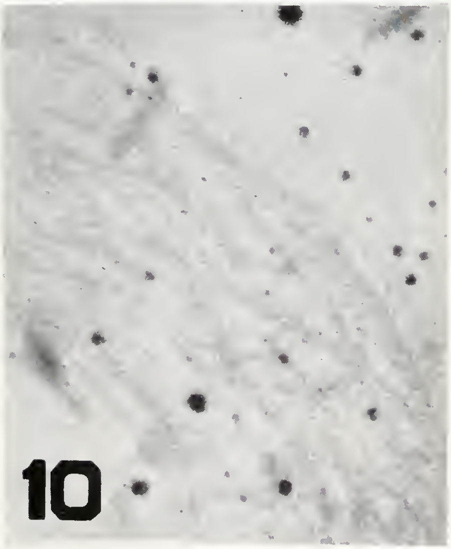
The agreement of the gross morphology of protozoans as revealed by electron micrographs with that seen by light microscope studies suggests that additional information may be gained by the electron microscope. The danger of artifacts introduced by the necessary treatment for such observations can be overcome by careful studies of morphological changes under different treatments and can be at least reduced to the level of that found in treatment preliminary to light microscope examination. The use of stains, in some cases specific, which are suitable to the optics of the electron system has been attempted, and will no doubt be developed to a satisfactory state. (Baylor, Appleman, Sears and Clark (1945) state that the electron "stain" must differentially alter the densities of various protoplasmic constituents.) The present day viewpoint of the necessity of knowledge of the specificity of a stain, and its action on well defined chemical constituents, ought to preserve the new field of electron microscopy from the array of sometimes meaningless colors now burdening light microscopy.

Needless to say, there is real need for careful identification of the specimens examined. Such was not possible at the time of this work. The necessity for pure cultures in investigations such as these is evident.









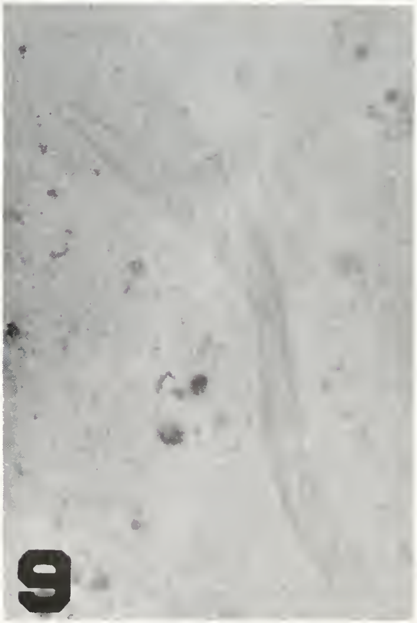


PLATE II

FIGURE 6. Two unidentified flagellates after osmic acid fixation (ca. 4,500 ×).
FIGURE 7. Osmic acid fixed ciliary flagellum, the shaft appears hollow (ca. 17,000 ×).
FIGURE 8. Fibrous flagellum broken away from the cell body (ca. 7,500 ×).
FIGURE 9. Fibrous flagellum showing rope-like arrangement of fibers (ca. 7,500 ×).
FIGURE 10. High magnification of frayed fibrous flagellum after osmic acid fixation (15,000 ×).

A mechanism of flagellar movement is suggested by the electron micrographs. The presence of fibrils in both types of flagella suggests that contraction and relaxation occurs as it is believed to occur in the fibers of muscle and connective tissue, that is, by molecular rearrangement. Contraction of individual fibers, particularly those arranged in the rope-like fashion seen in Figure 9, could account for the sweeping spiral movements observed in the living state. There is an indication that the fibrils of the fibrous type may extend into the cell body. Unilateral contraction of those in the cell body could certainly account for a variety of movements. The cilia of the ciliary type may function in either the original sweep or the recovery. They may also function in creating favorable currents in the water environment. In Figure 5 the cilia appear to be an extension of the protoplasmic membrane, and since no actual fibers can be observed within them, it may be that they serve to increase the sensory, absorptive or secretory surface of the cell.

The fibrous constitution of certain of the flagella indicates a broad distribution of fibers throughout the animal kingdom, from these simple organisms up through the complexities of the highly developed mammals. Are the fiber types the same throughout the whole kingdom? Is collagen, for example, present in all living animal forms, or does some other fiber take over its function in other species? Does the fiber form determine the function? Solution of these problems awaits further investigation and development of new and improved techniques.

SUMMARY

Certain flagellated protozoa have been photographed by the electron microscope. Two well defined types of flagella have been observed, a fibrous type in which the individual fibers occur in a twisted rope-like arrangement, and a ciliary type on which are arranged numerous small cilia. The shaft of the ciliary type in some cases appears to consist of fibrils. These observations verify in part and augment the masterly work of Harley P. Brown at The Ohio State University.

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THE ROLE OF SNAPPING SHRIMP (CRANGON AND SYNALPHEUS) IN THE PRODUCTION OF UNDERWATER NOISE IN THE SEA[1]

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INTRODUCTION

The sea has long been looked upon as a realm of silence. That this view is no longer tenable has been abundantly demonstrated as a result of extensive investigations with modern underwater sound detecting gear. These investigations, which during World War II became of grave importance because of submarine warfare and harbor defense, have shown that great stretches of numerous coastal areas are exceedingly noisy, periodically or perpetually, due to marine animals. Conversely, the investigations also indicate that vast areas of the deep ocean and of shallow water in higher latitudes are indeed notably silent.

The most widespread by far of all the biological underwater noises now known to exist in the sea is a crisp, high frequency, crackling noise caused by snapping shrimp. Examples of these animals are shown in Figures 1 and 2. It was not, however, until the second world war that the extent and persistence of this strange noise was realized and its cause determined. It has now become widely known as "shrimp noise" or "crackle."

The present report is intended to discuss, mainly, the cause and extent of crackle and to present the biological implications that explain its persistence and geographical distribution. Full details of the spectrum of the sound will be dealt with in another report (Everest, Young, and Johnson, in press).

EARLY HISTORY OF SHRIMP NOISE

Before discussing the recent investigations that led to identification of crackle, it will be instructive to review briefly some of the early references that no doubt pertain to this noise phenomenon.

1. Mariners operating small vessels in tropical waters have sometimes reported hearing strange crackling noises within the holds of their craft. The most common

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* Contributions from the Scripps Institution of Oceanography. New Series, No. 335.

[1] The work forming the basis of this report was done under contracts OEMsr–30 and NObs–2074 respectively with the Office of Scientific Research and Development (Section 6.1 NDRC) and the Bureau of Ships, Navy Department, while the authors were members of the staff of the University of California Division of War Research at the U. S. Navy Radio and Sound Laboratory, San Diego, California. The following organizations cooperated in the collection of much of the data upon which this report is based: Navy Department, especially the Bureau of Ships; Naval Ordnance Laboratory; the U. S. Navy Radio and Sound Laboratory (now the U. S. Navy Electronics Laboratory); the Scripps Institution of Oceanography; the Coast Guard; and the Columbia University Division of War Research.

explanation given was that the noise was caused by shipworms (teredos) working in the ship's timbers, or that it was due to superficial fouling growths on the hull. It is now known that the crackle from nearby shrimp beds is audible through the hull and probably forms the basis for these stories.

2. The U. S. Coast and Geodetic Survey encountered troublesome noises while engaged in radio acoustic ranging off Oceanside, California in 1933–34. The sound was described as being similar to static crashes or to coal rolling down a metal chute. No explanation could be found for the noise, though efforts were made to correlate it with meteorological and hydrographic conditions, and with road traffic on a highway adjacent to the coast (Comdr. O. W. Swainson, 1943, personal correspondence). Subsequent surveys have demonstrated that shrimp crackle is conspicuous in the area where these observations were made, and is loud enough in some spots to be audible to the unaided ear above the surface during calm water. Dredgings, also, showed the animals to be present in the shale bottom, which is honey-combed by molluscan bores.

3. A U. S. submarine operating in Macassar Strait off Balikpapan early in 1942 circled an area from which a strange crackling noise was emanating. Lt. Comdr. W. D. Wilkins, in reporting this experience, suggested that "the Japs may have some newfangled gadget that they drop." Snapping shrimp are common in the Balikpapan region and the type of bottom and depth of water off Balikpapan form a favorable habitat for them. Other reports from submarine officers strongly suggest that noises often encountered in coastal waters in lower latitudes are attributable to shrimp.

4. Sounds were heard through the hull of a small vessel in Beaufort Harbor, N. C. and in calm water off Cape Hatteras (Hulbert, 1943). These sounds were described as similar to that produced by "dragging a blackberry vine." No doubt they were produced also by snapping shrimp for these animals are abundant in Beaufort Harbor and are now known to be very noisy there. They also occur in offshore waters at Cape Hatteras (Hay and Shore, 1916).

To summarize the above as well as other data, it is apparent from what is now known that the crackling noise has been attributed erroneously to various other phenomena. Some of these are (a) static crashes, (b) rolling pebbles, (c) surf, (d) volcanic or terrestrial disturbances, (e) shipworms working in a wooden hull, (f) superficial fouling on the hulls of ships, (g) noises due to expansion or contraction of the ship's structure, and (h) clapping of oyster or clam shells.

The sharp "snap" produced individually by these shrimp when captured has long been known, and has often been heard during low tide in some areas. The sound has hitherto, however, been considered only a biological curiosity and not a source of significant underwater background noise. Biological sounds similar to shrimp noise will be discussed later.

Recent Investigations

Early in 1942 investigations of underwater ambient noise were begun by the University of California Division of War Research in the San Diego region. From the beginning of field tests a characteristic crackling noise of great magnitude was observed. It was first encountered at various locations off Point Loma and in the San Diego Yacht Harbor, and was found later in coastal waters off La Jolla, Oceanside, and other places on the southern California coast. The origin of the noise was a profound mystery until the end of 1942 when the cause was finally determined.

Identification of the noise was accomplished through laboratory tests of various animals and through field studies in many different habitats and over different populations. In the laboratory the animals were usually tested in large concrete tanks supplied with running sea water. Large public aquaria were also used.

For the listening tests and the exploratory studies, a simple crystal hydrophone and battery operated amplifier system sufficed. The equipment used for sound spectrum measurement in the field consisted of a non-directional hydrophone, a sound analyzer which was capable of selecting the various frequency bands (usually 50-cycles wide or a half-octave), and a graphic level recorder.

The sound emitted by an individual shrimp is a single sharp "snap" or "crack" produced only occasionally. It is the combined snapping of the members of a large population that results in a continuous loud underwater crackle over or near shrimp beds. In the audible band the crackling sound is comparable to the explosive noises produced by brisk burning of large quantities of dry twigs or frying of fat. Similar sounds can be heard by heterodyning ultrasonic bands in the 20–50 kc region down to the audible range. With increasing distance from the source, the sounds merge into a sizzle and finally a hiss without distinct cracks.

Sound spectrum analysis of crackle in the sea reveals that the shrimp noise has its strongest components in the higher frequencies. In contrast to shrimp noise, the noise produced by fish (Loye and Proudfoot, 1946, and Dobrin, 1947) is made up of low frequency sounds and is markedly seasonal and diurnal. In normal "water noise" resulting from waves and streaming of water, the maximum energy is also in the low frequencies. Because of this, shrimp noise is easily distinguished from both fish noise and water noise.

Figure 3 gives an idealized average of the sound spectra observed over shrimp beds in a variety of places. The ordinate for the sound measurements is sound pressure spectrum level.[2]

For comparison, the dashed curve shows the typical water noise observed in the deep sea, where there are no biological noises, for a state 1 sea.[3] This water noise increases at all frequencies with increasing roughness of the sea. It is apparent that at the lower frequencies the ambient noise over a shrimp bed is largely determined by the water noise found everywhere in the sea. However, above 2,000 cycles per second shrimp noise completely overrides the usual water noise. Above 10,000 cycles per second (10 kc) shrimp noise is some 30 db above the noise of a state 1 sea. The shrimp noise in the region of 10 to 20 kc exceeds the noise in a quiet sea by roughly the same amount as the audible noise in a factory exceeds the usual noise in a quiet residence without radio in operation. Because shrimp noise is greater than the water noise by such appreciable amounts, it is convenient to use an average spectrum level in the vicinity of 10 kc in studying noise suspected of being caused by shrimp. An average of the levels at 3, 5, and 10 kc has been used for this purpose, and also an average at 10, 15, and 20 kc; the latter has been arbitrarily selected for the present paper.

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[2] Sound level is given as a number of decibels (db) above some reference value which in the present work is a root-mean-square sound pressure of 0.0002 dyne/cm^2. This reference pressure is commonly incorporated in the calibration of sound-measuring instruments, but is frequently not explicitly stated. As a consequence, the decibel appears to be used with two meanings: a unit for an absolute measurement (with a reference value stated or only understood) and a unit for the *relation* of two sounds. In this paper when a sound *level* is stated in decibels it is in the first sense; the second sense is meant when a *change* or *difference* is given in decibels.

The decibel (db) is a logarithmic measure of power ratio (or ratio of pressure squared) such that a 100-fold increase in power (or 10-fold increase in pressure) corresponds to 20 db. A doubling of power is an increase of 3 db. A doubling of sound pressure is an increase of 6 db. The smallest change in noise readily perceptible to the ear is of the order of 1 or 2 db.

The noise being dealt with here is conceived to be comprised of a rather continuous distribution of sound vibration frequencies. The words "pressure spectrum level" refer to the effective sound pressure level for that sound energy contained in a band 1 cycle per second wide centered at the frequency indicated.

[3] The "sea" as used here refers to waves which are still growing because of the wind, and as such represent an additional motion superimposed upon the swell. A state 1 sea corresponds to waves from crest to trough less than 30 cm. high. See *Instructions to Marine Meteorological Observers.* U. S. Weather Bureau No. 1221, June 1941, p. 54.

Analyses were made, also, of single shrimp snaps by taking oscillograms of the sound from isolated identified specimens. The shrimp were placed in a gauze net within a rubber bucket filled with sea water. The whole assembly was lowered into a fresh water lake, then the net was drawn from the bucket by means of a cord. Contact with fresh water stimulated the shrimp to snap and photographs were made of the snap impulses shown on the oscillograph screen.[4] The whole snap lasts about ½ to 1 millisecond. Further details are to be found in the acoustical report (Everest, Young, Johnson, in press). It is sufficient to note here mainly that the spectrum of a single snap, derived by a Fourier integral analysis, agrees qualitatively with the average spectrum over a shrimp bed.

It is not possible to reproduce in the laboratory the extent of crackle heard over shrimp beds in nature, or to simulate the hiss heard at a distance from the bed. Therefore, corroborative evidence must come from correlated field observation and spectrum measurements as discussed later.

The explanation of the local underwater sound mystery shed light on a number of qualitative reports of the type discussed in the previous section. Ultimately, it made possible the prediction of this type of ambient noise in other areas.

Sound surveys made since 1942 in widely separated areas along the East and West coasts of the United States from Key West to Chesapeake Bay and San Diego to Puget Sound, in the Bahamas, and in the Central and Southwest Pacific have confirmed the predicted, widespread distribution of shrimp crackle in localities that are ecologically suitable for snapping shrimp, and its absence in other situations. In the following section an explanation of the relationships is given.

The Biology of Snapping Shrimp and Its Relation to Sound Levels

The geographic locations of noisy crackling areas in the sea and the stability of the sound in these areas are so closely bound to the snapping habit and to the living requirements of these animals that it is necessary to consider their biology in some detail in order to elucidate these problems.

Biological relationships

Technically, the snapping shrimp are related to the commercial shrimp, which they resemble in general appearance. In life habits and details of structure, how-

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[4] The assistance of T. F. Johnston and T. McMillian in this phase of the work is gratefully acknowledged.

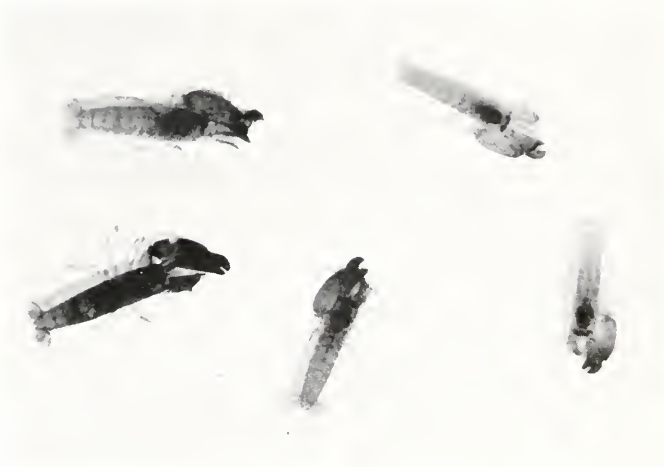


FIGURE 1. *Synalpheus lockingtoni* natural size. Photographed from live specimens. At upper left: specimen with claw open to snap.

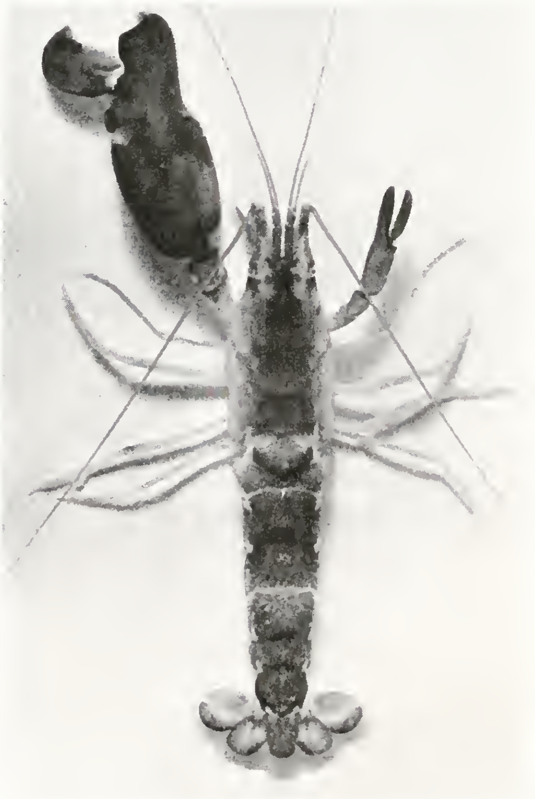


FIGURE 2. *Crangon californiensis* 2 ×.

ever, they are very unlike the common shrimp. The snapping shrimp family Crangonidae (Alpheidae, see Rathbun, 1904) comprises about 27 genera and numerous species. Of these only the species of two genera, viz., *Crangon* (also called *Alpheus* by many authors) and *Synalpheus,* are capable of vigorous snapping. In the literature there are recorded about 215 species of *Crangon* and 150 species of *Synalpheus.* Two species of these genera are shown in Figures 1 and 2.

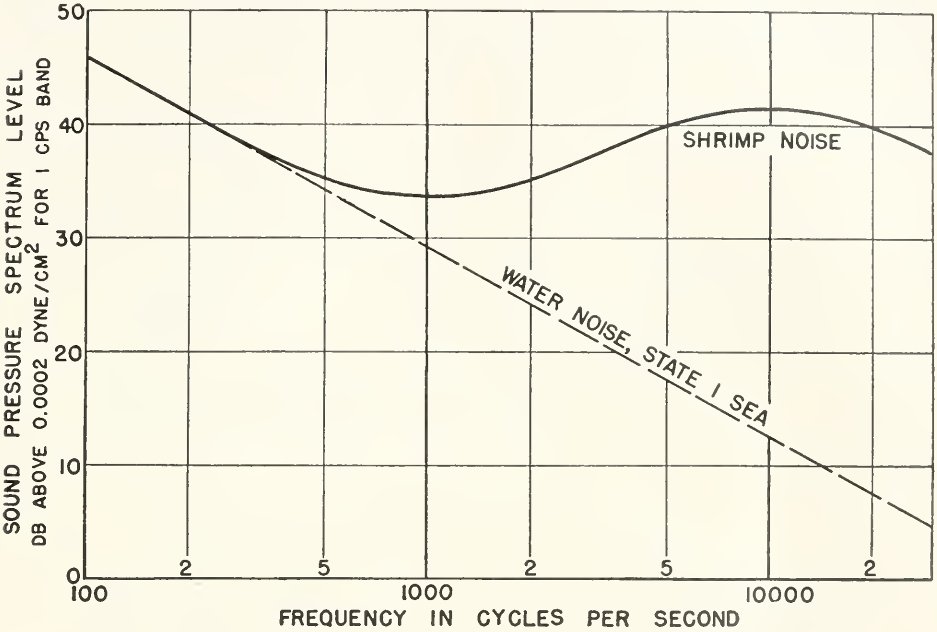


FIGURE 3. Average ambient noise spectrum over shrimp bed.

The snapping habit

Studies of most species of these two genera have been based on preserved specimens; consequently, the snapping has not been observed directly in many species. The claw structure, however, indicates that all the species do snap to some extent. The species range in size from about 2 cm. to a giant species, *C. strenuus,* attaining a length of 8 cm., but it is clear that size is not necessarily correlated with the noise produced. The few direct sound measurements available of isolated specimens indicate, for example, that *Synalpheus lockingtoni* may produce a louder snap than its larger relative *Crangon dentipes.* The average peak pressure level of *Crangon* for a distance of a meter was 115 db above 0.0002 dyne/cm.^{2}; whereas it was about 124 db for *Synalpheus.* While the difference may be real, the present observations are too few, and the spread too great, to confirm this.

The habit of snapping is associated with defensive and offensive activities. In closing the snapping claw, a vigorous jet of water is produced by means of a plunger arrangement described below. This sudden gush of water serves to frighten away enemies approaching too near. The antagonist may also be driven away or sometimes killed by a direct blow of the small hammer, but the biological significance of the accompanying loud sound is uncertain.

The snapping mechanism

A good deal has been written regarding the sound-producing mechanism, especially by Coutière (1899), Brooks and Herrick (1894), and Volz (1938). This mechanism consists of one disproportionately large hard appendage comparable to one of the large "claws" of the lobster. Figure 4 shows an enlarged drawing of the snapping claw of *Crangon californiensis,* a vigorous snapper (*cf.* Fig. 2).

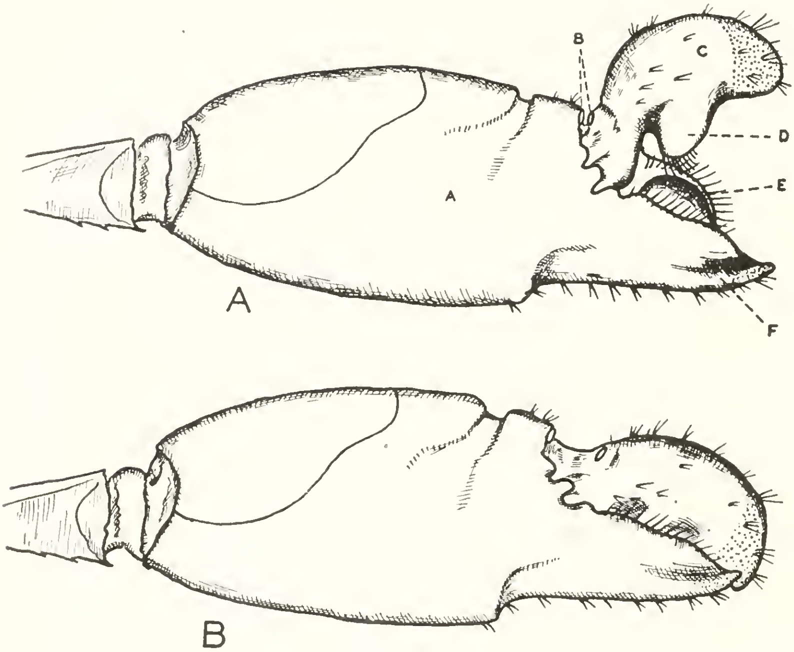


FIGURE 4. *Crangon californiensis.* Snapping mechanism. *A*, Snapper open; *B*, Snapper closed —A, palm; B, suckers; C, movable finger; D, plunger; E, socket; F, thumb.

In some species, the snapping claw is nearly as large as the body of the animal, giving ample room for development of strong snapping muscles. A movable hard "finger" (C) is jointed near the outer end of the "palm" (A) of the claw forming a forceps-like arrangement with the immovable "thumb" (F). On the inner side of the finger is a plunger-like structure (D) which fits into a socket (E) in the thumb. Leading forward from the socket is a groove through which the water escapes when the plunger is suddenly forced into the hollow. When the finger is raised, the plunger is withdrawn from the socket, and it has been held by some early observers that the noise is produced by this action, similar to the "pop" heard when a cork is suddenly pulled out of a bottle (Wood-Mason, 1878). The biological function of the plunger-socket arrangement, however, is the production not of sound, but of the water jet mentioned above. It may aid, also, in preventing dislocation of the finger when it is violently snapped.

The plunger-socket device is present in both *Crangon* and *Synalpheus*. In all species of *Crangon,* there is also a "sucking" disc on the outer side of the finger near its point of attachment (Fig. 4A). When the finger is fully raised, this disc contacts precisely a similar disc on the palm so that the two are firmly in contact when the finger is in position to snap. These suckers serve as a trigger to hold the finger back, and extra muscular tension must be exerted in breaking the contact. This in turn increases the force of the impact. The suckers are absent in *Synalpheus,* but in the same relative position there are smooth surfaces that contact perfectly. These surfaces probably serve the same purpose as the suckers. That the suckers on the claw of *Crangon* do serve as a restraining trigger is readily demonstrated. When the finger of a dead specimen is forced back to its fullest extent, it is held there so firmly that some force is needed to break the contact. This was demonstrated even in a specimen that had been preserved in formaldehyde for several months.

The precise way in which the sound is produced is still a matter of some doubt. The best explanation appears to be that the sound is produced by impact as the movable finger strikes a glancing blow on the opposing tip of the thumb. The tips of these structures are either very heavily calcified as in many species of *Crangon,* or are tipped with a heavy formation of chitin as in *Synalpheus lockingtoni.*

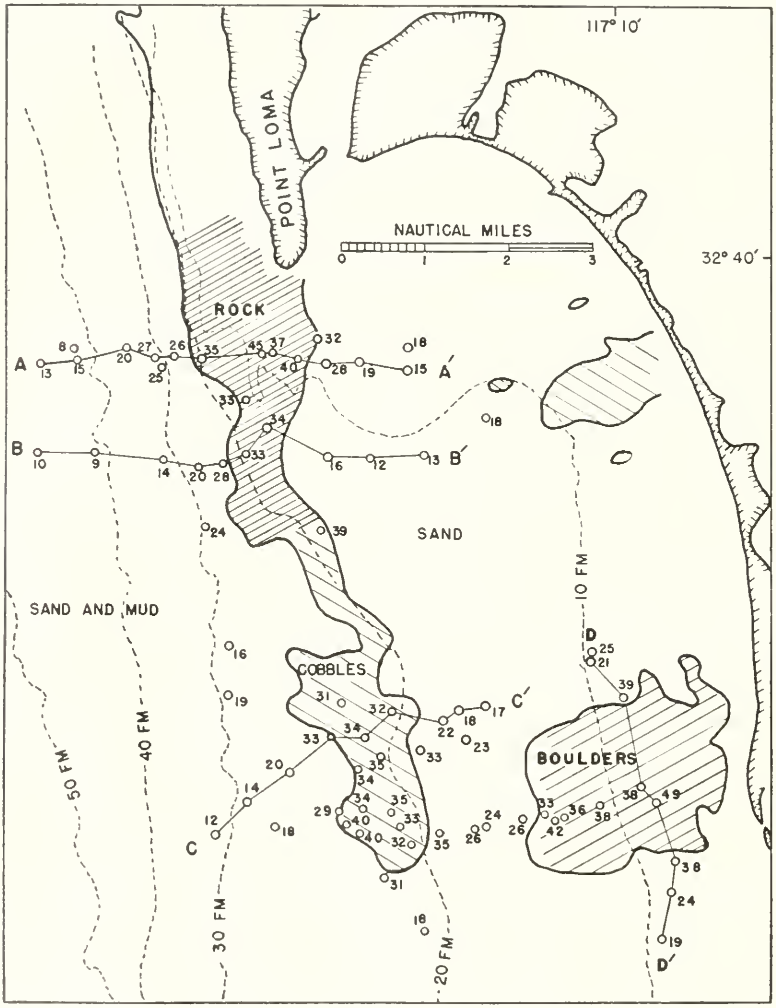
In a painstaking investigation of *Crangon dentipes* and *Synalpheus laevimanus* of the Mediterranean, Volz (1938) found a small chitinous ridge at the inner upper margin and back wall of the socket. This ridge, he concluded, is part of a breaking device which incidentally aids in producing the sound when it is struck by the plunger as it forcibly enters the socket.

It is of passing interest to note that the snapping claw may be either right or left; if it is lost through injury the inconvenience is only temporary, because a new one is promptly grown in the first or second moulting when the shrimp sheds its old shell. In this process of renewal, however, the original large claw is replaced by a small one, while the former small pinching claw is enlarged and becomes the new snapping claw (Wilson, 1903).

Type of habitat and crackling areas

The species of *Crangon* and *Synalpheus* are bottom-living animals living mainly below zero tide level and, though capable of swimming, they rarely do so in the adult stage. They are notably secretive and demand ready-made or easily maintained burrows. Hence, they seek concealment in crevices and holes provided by coral, stones, shells, calcareous algae, and other solid objects. It has been demonstrated repeatedly by collectors that they live preponderantly on these bottom types. This habit renders collecting very difficult in most instances, especially when a dredge must be used. Hence the animals are far more abundant than generally realized.

In the East Indies they are especially fond of bottoms covered by the calcareous alga *Lithothamnion* (de Man, 1916). They commonly live among the roots of eel grass and holdfasts of seaweeds and among mussels, barnacles, and other substantial fouling organisms on wharf piles, etc. Some live in the channels and pores of living sponges. In many of these situations there can be heard a continuous fusillade of snapping. Where sound measurements have been made at old heavily-fouled piers, the spectrum level in the region of 3 to 10 kc has reached about 60 db above 0.0002 dyne/cm^2.


117° 10'
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POINT LOMA
NAUTICAL MILES
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49
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D'

FIGURE 5. Locations of series of stations occupied for sound level measurements across areas ecologically favorable to snapping shrimp. The number adjacent to each station is the average of the 10, 15, and 20 kc spectrum levels.

Snapping shrimp do not normally live in any significant numbers on extensive sand or mud bottoms without some sheltering materials. However, some species (for example, *Crangon californiensis*), tolerate muddy conditions among eel grass, etc., and have been dredged with mud, particularly in harbors littered with solid debris. Crackling is intensive in some of these situations. A European species, *Crangon ruber,* is said to be "not uncommon" on the mud bottom off Ram's Head near Plymouth, England and in the Mediterranean. No information is available regarding the underwater sound produced by this species.

The way in which the crackling noise increases over a habitat favorable to shrimp is indicated by Figures 5 and 6. Figure 5 shows some of the stations in the San Diego area at which sound spectrum measurements were made under careful control. The number adjacent to each station is the average of the 10, 15, and 20 kc spectrum levels. Water depths may be estimated from the depth contours. The bottom character here is well established.[5] There is a clearly defined stretch of shale which is well honey-combed by abandoned mollusk borings in which the shrimp find retreats. Continuous with the shale is a less regular stretch of cobbles. Nearby is an isolated patch of boulders. Dredgings reveal all these to be populated by shrimp in contrast to dredgings from the surrounding sand-mud and sandy areas of comparable depths.

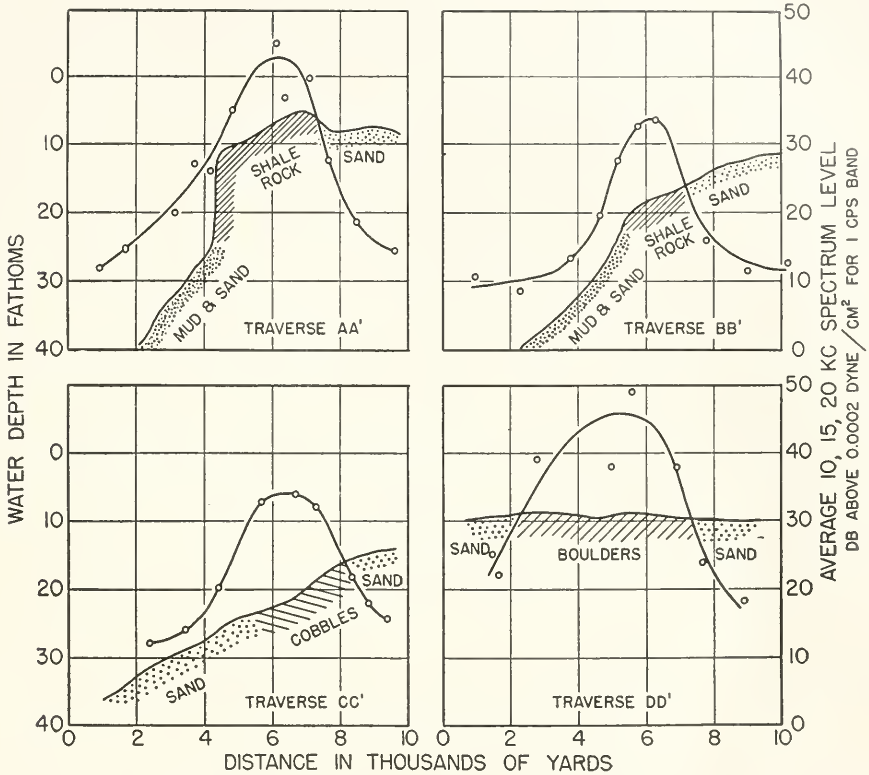

FIGURE 6. Profiles of bottom at stations occupied in Figure 5 showing spectrum levels across shrimp beds.

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[5] The assistance of Dr. F. P. Shepard in these studies is gratefully acknowledged.

The sound levels between the cobbles and boulders are higher than would be expected over sand, but this apparently results from a combination of sound transmitted from both areas since the positions are well within the sound range of both.

Specific traverses are indicated by *AA′*, *BB′*, *CC′*, *DD′* in Figure 5. The corresponding depth profiles (use left-hand scale) are given in Figure 6 along with the average 10, 15, 20 kc spectrum level (right-hand scale) at each station.

These show how markedly the sound, in the frequency range 10–20 kc, rises in passing over the shrimp habitats. During low sea states, the transmitted shrimp sound is appreciable to a distance of over a mile from the boundary of the bed.

Similar sound spectrum measurements were made at numerous shallow and deep water stations at widely scattered localities on the east Pacific coast, among the Pacific Islands [6] and along the west Atlantic coast and the Bahamas.[7] A typical set of observations, from six stations off Barbers Point, Oahu Island, is given in Figure 7. The coral, algae, and shell bottom extends into deep water, yet the sound level falls off rapidly in depths greater than 30 fathoms, despite a bottom type favorable to shrimp. At the outermost station the shrimp noise presumably merged with the normal ambient water noise.

An average 10, 15, 20 kc spectrum level of 30 db was arbitrarily chosen as a dividing point for sorting the observations, values greater than this being designated "high" and those less as "low." When the data from both the Pacific and Atlantic were studied with regard to bottom type, it was found that within 0–30 fathoms about 80 per cent of the "high" levels were observed over bottom types favorable to shrimp. In contrast, about 90 per cent of the "low" sound levels (below 30 db) for this depth zone were over unfavorable bottom. In view of the difficulty of establishing precisely the character of the bottom over considerable areas, this correlation is surprisingly good.

Depth distribution

Data on hauls of snapping shrimp, taken at various depths, are scattered widely throughout the biological literature (de Man, 1916). Table I shows how 185 species of *Crangon* and *Synalpheus* have been reported as occurring in five depth zones. The table includes only trawling and dredging collections from the bottom.

TABLE I

Distribution of 185 species of Crangon *and* Synalpheus *reported from 5 depth zones*

Bottom depth zone	Number of times shrimp reported	Number of species
0–30 fathoms	418	171
30–70	101	59
70–100	5	4
100–250	14	9
Over 250	7	4

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[6] Many of these field data were collected by W. E. Loomis of the Naval Ordnance Laboratory during a survey in cooperation with that organization.

[7] These observations were made in cooperation with the Columbia University Division of War Research, Bureau of Ordnance and the Naval Ordnance Laboratory.

While most of the species are reported in 0–30 fathom water, it cannot be concluded that Table I gives the actual distribution, since it is not possible to know precisely how uniform the effort and efficiency of collecting have been in various zones. Presumably, it has been roughly comparable with respect to the number of hauls in the first two zones. This is supported by a summary of 3,483 hauls by various expeditions that have contributed to the study of these animals. Among these expeditions are especially those of the USS "Albatross" (1883–1911), the "Siboga" (1899–1900), the "Percy Sladen" (1905), and the "John Murray" (1933–1934). An analysis shows that 17 per cent and 20 per cent of the hauls were taken respectively in the 0–30 fm. and 30–70 fm. zones. The remainder were in deeper water. Thus the table gives a fair idea of the depth distribution and indicates that the largest populations are in the 0–30 fathom zone.

Acoustic data support this belief, for in water deeper than 30 fathoms the sound levels are low (except where sound is transmitted from nearby shrimp beds), even though the bottom type is favorable for shrimp, as in Figure 7. It is probable that the deeper ranging species have their maximum concentration in depths less than 30 fathoms, and that the typically deep water forms do not occur in sufficient concentrations to produce a continuous crackle like that heard in shallow water.

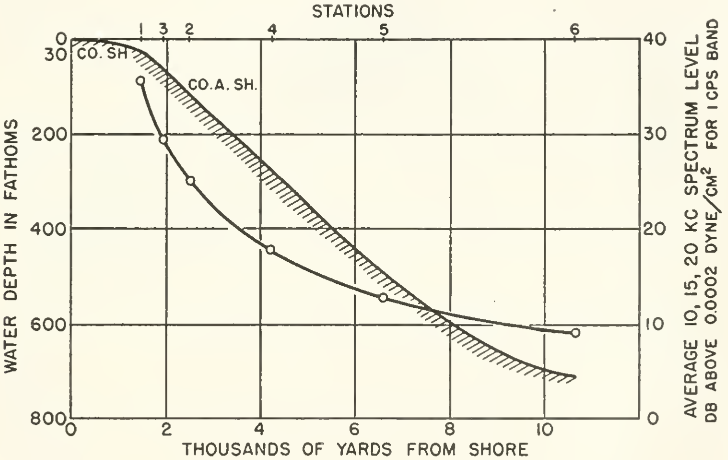

FIGURE 7. Sound observations from Barbers Pt., Oahu showing drop in spectrum level with depth of water and distance from shallow (less than 30 fm.) areas favorable to snapping shrimp.

Life history and its bearing on continuity of crackle

The bedlam of crackling noise over a shrimp bed is never ending. This continuity is correlated with the life history and stability of shrimp populations. The fertilized eggs of snapping shrimp are carried by the adult female until they hatch as free-swimming larvae about 4 mm. in length. After a period of drifting with the water currents, the larvae settle to the bottom and assume the structural characteristics and habits of the adults. No snapping is possible until this time. The free-floating period serves to disperse the animals widely and they thrive after settling to the bottom where living conditions are favorable for adults. From what is known of a few species, there appears to be a long breeding season in low latitude waters. Gravid specimens have been found in all seasons at La Jolla. Near the higher latitudes the breeding season is probably shorter.

The duration of life of the individual is not known, but adults collected at La Jolla, California, in December 1942, and January 1943, were still thriving in an aquarium after twenty-two months of captivity. These facts are mentioned because the maintenance of overlapping generations is, no doubt, important in explaining the seasonal continuity of the crackling sound. A short breeding season and a short span of life would result in a considerable variation of the adult population and this, in turn, would be expected to cause an appreciable seasonal variation in shrimp noises. No seasonal variation of this nature has yet been noticed.

Observations have not revealed any swarming or migratory movements such as characterize many other marine animals during the breeding season, and this, too, is correlated with the small fluctuations in sound level.

The snapping shrimp are gregarious to the extent that large numbers of a given species are found either in pairs or as solitary specimens in isolated retreats within the area. The occupants of these retreats make a noisy protest when an intruder approaches. Specimens have been observed to leave their burrows for short journeys in search of food, especially at night, but those under observation always returned to their respective retreats. These retreats in some instances were occupied steadily by the same specimen for several months. *Crangon californiensis* showed a much stronger tendency to desert its burrow than did other local species observed.

There is a small diurnal variation in shrimp noise. At night the levels are 2 to 5 db higher than in the daytime. In addition, there is a slight peak in the noise level shortly before sunrise and after sunset. The effect is caused, probably, by increased activity of the shrimp at these times. In the laboratory tanks the shrimp appear to increase their search for food at night. This is also a well-known behavior among many other marine animals. Thus, the overall increased activity would lead to more irritation and more snapping.

It should be noted that an increase in sound level of 3 db actually represents a two-fold increase in shrimp activity.

Observations made so far have indicated no appreciable seasonal variation in shrimp noises. This is in agreement with the known stability of the adult population discussed above. This result may not apply near the edge of the shrimp belt, however, or at unusual localities inside the belt having large seasonal variations in water temperature.

Geographical range of shrimp correlated with distribution of crackle

The species of *Crangon* and *Synalpheus* are confined to coastal or shallow water throughout the tropical and subtropical regions. This type of distribution is, no doubt, governed by water temperature, but precisely how this operates is not yet clear. In general, the 11° C. winter surface isotherm marks the approximate northern and southern limits of their continuous range (Fig. 8). In Figure

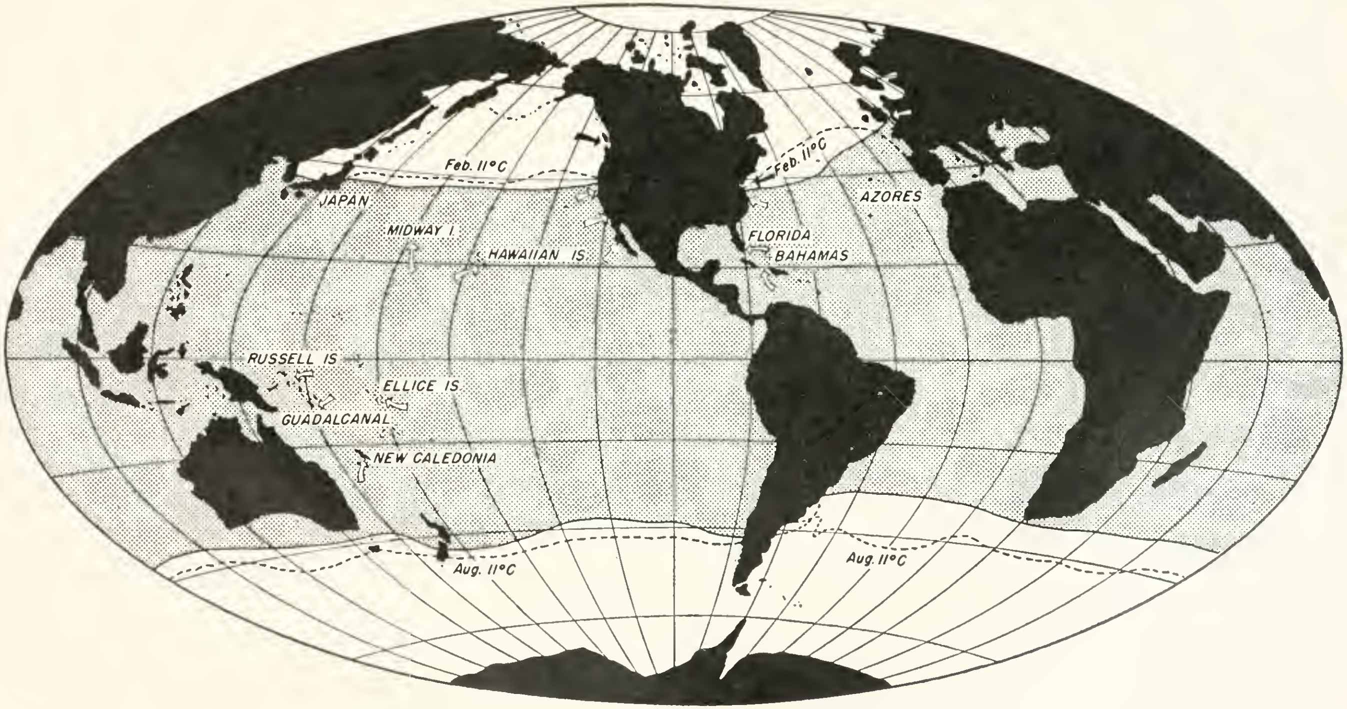
Feb. 11°C
Feb. 11°C
JAPAN
AZORES
MIDWAY I.
HAWAIIAN IS.
FLORIDA
BAHAMAS
RUSSELL IS.
ELLICE IS.
GUADALCANAL
NEW CALEDONIA
Aug. 11°C
Aug. 11°C

FIGURE 8. Geographical range of snapping shrimp (*Crangon* and *Synalpheus*). These genera are found within the shaded area where depth and bottom conditions are favorable. The arrows denote general areas where ambient noise has been measured.

8 this range is represented by the shaded area. The thermal control of distribution must, however, be a complex one. It is not accomplished simply by the temperatures falling to or below this level during the winter. Certain critical periods of life such as spawning and larval development appear to require a considerable period of warmer temperatures, near or above 17° C. during the summer. The isolated populations lying outside of the 11° C. isotherms are probably governed by favorable local conditions. Examples of this sort are seen at the north end of Honshu, near Vladivostok, and along the coast of Ireland. A comprehensive study of the literature giving the localities where snapping shrimp have been collected one or more times, shows them to be present in all tropical and subtropical locations where extensive surveys for crustacea have been made. In the strictly tropical and subtropical waters, the distribution of shrimp is believed, therefore, to be continuous within the areas where depth and type of bottom are favorable. This agrees with the available acoustic data from sound surveys made in various parts of the world.

There is but little quantitative information on geographical distribution. The animals are, as a rule, difficult to collect in representative numbers because of their habits of seeking retreats in hollows and crannies of rock, coral, etc. It seems clear, however, that the greatest number of individuals as well as species occur in tropical waters among coral and other reef organisms. The northern and southern boundaries of continuous distribution along the open coast are, no doubt, transition zones in which the populations become progressively sparser, but how wide these zones are is not known. However, the animals are known to be quite abundant at Beaufort, N. C. and at Monterey Bay, California, and listening tests in these areas reveal shrimp crackle to be pronounced, though spectrum measurements are not available. The region between Cape Lookout and Wimble Shoals on the east coast and the region of San Francisco on the west coast represent the border of distribution (Hay and Shore, 1916; Schmitt, 1921). Sparse crackle has been heard in these regions, but sound measurements fail to show the characteristic spectra of shrimp noises. Acoustically, there must be a certain minimum concentration of shrimp in a given area before the snapping sound can materially influence the reading of instruments which require an appreciable time to respond. Sound measurements from latitude 9° S. to 33° N. indicate about the same levels over shrimp beds irrespective of latitude.

Biological Sounds Similar to Shrimp Noise

Among the known animals producing noises that may be confused with shrimp crackle or that may in a minor way contribute to the ambient noise are the following:

Gonodactylus oerstedii (and no doubt other species of this genus), a crustacean generally known as "mantis shrimp" or "squillid," makes a clicking sound when striking out with its claws. Like the snapping shrimp, this animal has a wide tropical distribution and lives in similar habitats.

At least two species of the *Coralliocars* shrimp, *C. graminea* and *C. wilsoni,* are capable of snapping by means of a structure similar to that used by regular snapping shrimp. They are not known to be abundant and apparently have a geographic and habitat range falling within that of the snapping shrimp.

Typton spongicola of the Mediterranean and *Pontonia pinnae* of East Africa are species of crustacea said to be capable of snapping. They are not considered numerous.

The larger crabs such as *Cancer* and *Portunus* have been observed to make noises sounding like the individual crack produced by *Crangon* and *Synalpheus*, but the noise is incidental to the cracking of brittle shells of small clams, etc., for the food within the shell. Crackling from this source can, of course, be present only as long as shell food is being eaten. Other animals, including fishes, which occasionally crack shells for food would also fall into this category of incidental noise makers. To produce the volume of crackling that occurs continuously over shrimp beds would, however, very quickly exhaust all available shells. Hence cracking of shells is believed to be a very small contribution to the ambient noise.

The trigger fish is said to be capable of making a clicking sound by means of the joints of some of the fin-spines; the mackerel by means of its pharyngeal teeth.

Populations of barnacles produce very weak crackling sounds, barely audible at very close range. Barnacles and perhaps other larger crustacea (preening their shells or feeding) appear to be the main cause of these very faint noises sometimes heard when the hydrophone is within a few feet of the shore in quiet waters north of the geographic range of snapping shrimp.

In the Hawaiian area, a very troublesome raucous noise with a sharp high peak at 3 kc has been encountered. This has been called "evening noise" in view of its regular occurrence each evening between about 7:30 and 10:00 P.M. In one harbor, this caused a 10 db rise in overall level of the ambient noise during the evening hours. The origin of the noise was not determined, but the possibility of its being somehow associated with increased shrimp activity at a distant bed in the evening has been suggested. However, an obvious difference in character between shrimp noise and "evening noise" militates against this explanation.

Conclusions

1. The sea is far more noisy, as a result of biological noises, than was commonly believed.

2. The most widespread of the biological underwater sounds is a continuous high frequency crackle caused by snapping shrimp (*Crangon* and *Synalpheus*). The continuity of high magnitude sound produced by populations of these animals appears to have no counterpart in biological nature.

3. All shallow water areas (0–30 fathoms) within the geographical range of *Crangon* and *Synalpheus* and with bottom conditions and temperatures favorable to these animals are likely to be noisy.

4. The strongest components of shrimp noise are in the frequency range 2 to 15 kc.

5. The sound levels over snapping shrimp beds are usually 30 db higher than state 1 sea noise.

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BEHAVIOR OF THE CELL SURFACE DURING CLEAVAGE. VII. ON THE DIVISION MECHANISM OF CELLS WITH EXCENTRIC NUCLEI

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The conventional diagram of a typical cell shows the nucleus in a central location, but actually in many cases the nucleus does not occupy the exact geometrical center of the cell. The present paper deals with the mode of division of cells with excentric nuclei, especially in connection with the general theory of the mechanism of cell division proposed in a previous paper of this series (Dan, K., 1943b).

Review of the Proposed Theory

The salient features of the theory are as follows: (1) The asters of sea-urchin eggs are spiny spheres with numerous gel rays radiating out in all directions. (2) Cleavage is brought about as a result of the pushing apart of two such radiate spheres by the autonomous elongation of the spindle (Fig. 1). Applying this concept, a cell in the resting stage can be regarded as a mass of fluid cytoplasm contained in a gelated cortex. When two radiate gel spheres which have developed and reached their maximal size within this fluid cytoplasm are pushed apart by the spindle, the original fluid mass will be divided into two portions which are surrounded externally by the cortex and supported internally by the spiny spheres (Fig. 1). Observations on adhering kaolin particles have shown that the surface of the furrow expands during the latter half of the division process (Dan, Yanagita and Sugiyama, 1937; Dan, Dan and Yanagita, 1938). We can account for this expansion phase if we visualize a definite amount of fluid cytoplasm being divided by the above mechanism. As the asters are pushed apart, the cortical layer covering the cleavage furrow will be sucked in between them and will show a linear stretching along the sides of the furrow. But actual observations have also shown that, at the beginning of the cleavage process, before the expansion phase, the furrow surface undergoes a phase of shrinkage. This initial shrinkage phase may be explained by two more details of the division process. (3) The astral rays cross at the future cleavage plane (Fig. 1, Stage 2), and (4) the rays are anchored to the cortex of the cell. As was fully discussed in the previous paper (Dan, K., 1943b), the rays exert a traction effect upon the cortex of the egg and this effect causes the initial shrinkage, whereas the later expansion of the furrow surface is due to the suction effect mentioned above. Let us consider this more fully. If the underlying spindle elongates while the tips of the individual rays are attaching to the cortex, the loci along which the tips of the crossing rays will move are practically the same as the loci of the apices of triangles made by the crossing rays, the spindle (i.e., the base of the triangle) and a line connecting the tip of each ray with the astral center of the opposite blastomere. Such loci form a curve directed toward the base line and gradually approach-

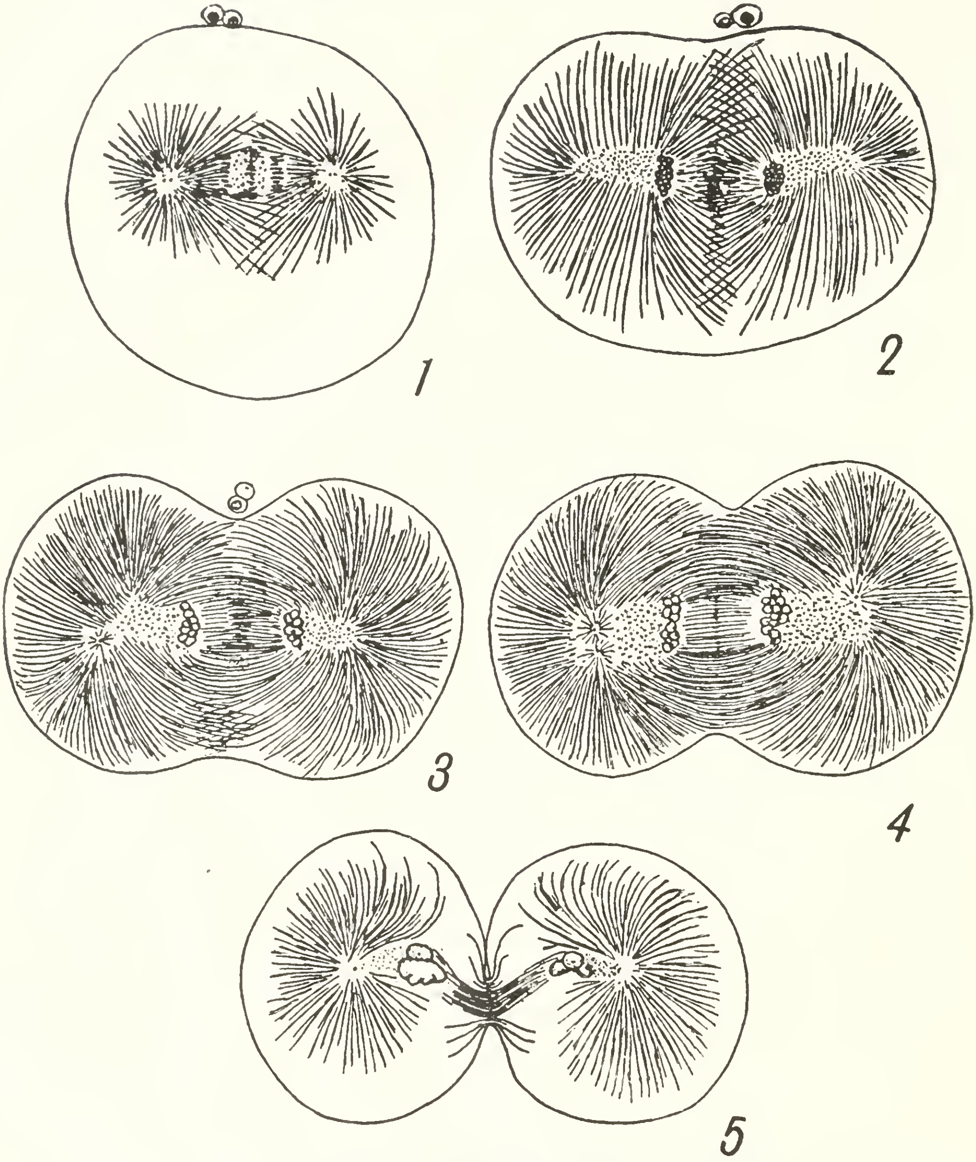

FIGURE 1. Division of the eggs of *Cerebratulus lacteus.* Reproduction of Yatsu's Text Figure C. Legends from Yatsu. "Five stages of the first cleavage, × 400. 1. Anaphase of the first cleavage mitosis. Crossing of rays, and the position of the spindle. Difference in distance between two centrioles at either end. 2. Telophase of the first cleavage mitosis. The centrosome has enlarged and been bent downwards. The crossing of rays has begun to loosen. A faint indication of fountain figure is seen at the pole regions. 3. Telophase; a vertical section through the poles and a centriole. Constriction has begun on both the animal and vegetative sides. Sheath spindle has been formed. Fountain figures at the poles of the spindle have become more distinct. 4. Telophase, a horizontal section through three centrioles (about the same stage as 3). Sheath spindle is very well formed. Centrosome has greatly enlarged. The distance between the rows of karyomeres is approximately the same as the original length of the spindle. 5. Late telophase (a vertical section). Sheath rays have been formed. Fountain figure in the equatorial rays."

ing a vertical line bisecting the base (Fig. 2). As the triangles become less equilateral the curve becomes greater. On the contrary, if two astral rays meet exactly on the median plane an equilateral triangle is obtained and the locus of the apex coincides with the vertical line bisecting the base. Seven such loci are shown in Figure 2. As an example, if a pair of symmetrically crossing rays is considered, two unequilateral triangles which are mutually mirror images are obtained and their apices will converge toward the mid-line as the spindle elongates. This means that the surface included between the apices will be made to shrink. The situation is most clearly demonstrated by an experimental model, shown in Figure 3. Since both the traction effect of the astral rays and the suction effect in the cortical layer of the furrow are equally direct results of spindle elongation, there is no contradiction of the statement made under (2).

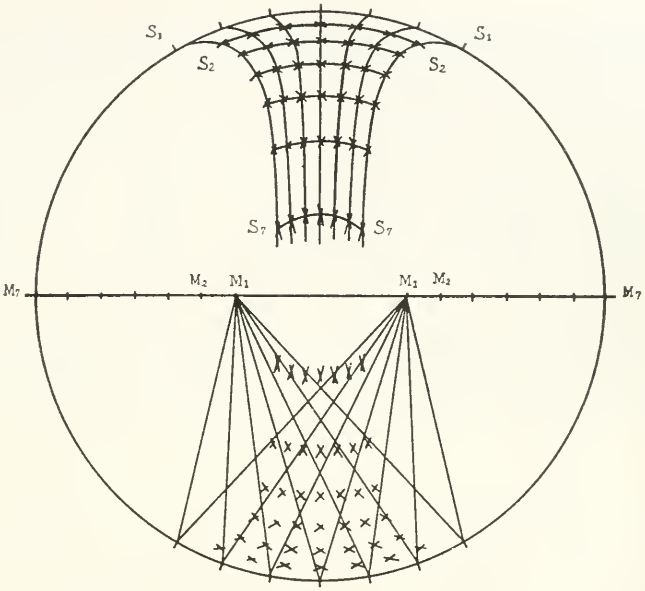

FIGURE 2. Loci of seven pairs of rays which meet end to end and which are attached to the cortex when the spindle elongates. The lower half of the figure shows the positions of the tips of the rays for seven different lengths of the spindle such as are indicated on the horizontal line. The upper half shows the same groups of points in terms of the surface. Notice that the rate of shrinkage of the surface is fast in the beginning and slow later, while the rate of the inward shift of the surface is slow at the beginning and fast toward the end. Note also that the wider the range of crossing of the rays, the greater is the rate of shrinkage. The innermost position of the surface (S_7S_7) indicates that a complete division of the cell cannot be achieved by this mechanism alone.

Finally it must be considered how and when the traction effect is relayed over to the suction effect. To explain this another feature of the division figure must be examined. (5) A fountain-figured bending of the polar rays takes place (Fig. 1, Stages 3 and 4). If the rays were rigid and not pliable, as soon as the spindle elongated the suction effect would be felt at the cleavage plane and there would be no time for the traction effect to play its role. But actually the rays are pliable. Therefore when the spindle elongates and pushes the asters against the polar surfaces, the rays yield to the pressure and bend in a fountain figure. By this bending of the rays, the spindle elongation is not transmitted directly to the cell contour but is buffered. Once the bending of the polar rays reaches the maximal degree, however, further lengthening of the spindle brings about the suction effect. Thus, even though the dynamic cause of cell division is single—namely, the spindle elongation—its effect on the cell surface involves two factors which come into play in succession. It may be pointed out in passing that the fountain-figured bending of the polar rays theoretically ought to bring about an expansion of the overlying surface (polar surface). As a matter of fact, the polar surface does expand from the beginning of the cleavage process, in contradistinction to the furrow surface.

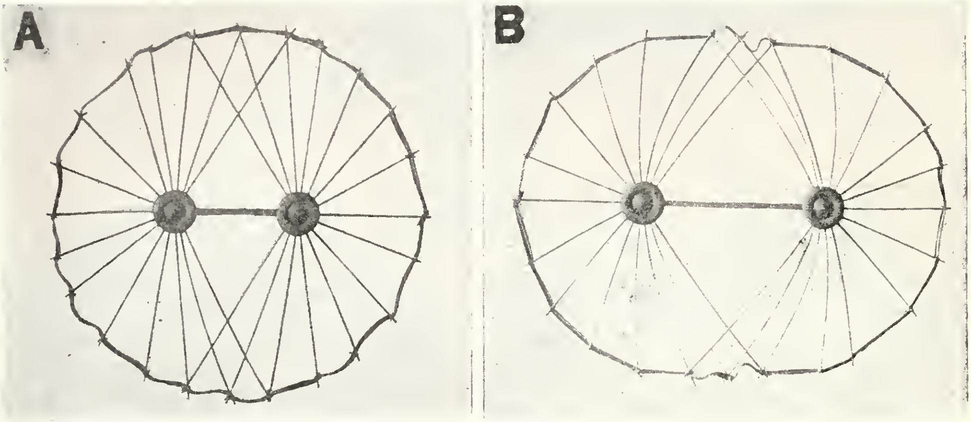

Figure 3. A model imitating the initial shrinkage phase of a cell with a central spindle. Astral centers are represented by wooden wheels in which bamboo rods are firmly planted imitating astral rays, which, in turn, are tied to a rubber band simulating the cell membrane. A wooden bar which represents the spindle is also firmly fixed to each wheel, the bars from the two wheels overlapping in the spherical condition (*A*). The bars have small holes along their lengths so that they can be fixed in any desired length by putting a pin through these holes (*B*). In *B*, note (1) the flattening of the furrow regions, (2) the shrinkage of the furrow surface, (3) fountain-figured bending of the polar rays and (4) consequent stretching of the polar surface. Note also that (5) the spindle is straight after the elongation.

THEORETICAL CONSIDERATION OF THE DIVISION MECHANISM OF CELLS WITH EXCENTRIC NUCLEI

Returning to the division of cells with excentric nuclei, one might say that there are only two known facts which are widely accepted today. One is that the mitotic figures resulting from excentric nuclei are also situated excentrically in the cells. The other is that the cleavage furrow appears first at the side of the cell nearest to the excentric mitotic figure, later spreads around the two sides and finally completes its circuit at the opposite side of the cell from that at which it was first formed (Ziegler, 1898).

In order to repeat the observations, the authors used the eggs of the sand-dollar, *Astriclypeus manni.* In these eggs the mitotic figures are excentric toward the animal pole, and the furrow starts to appear from there. The astral rays are much better defined than in other forms. They are clearly seen crossing all along the median plane despite the fact that the rays going to the animal hemisphere are shorter than those distributed in the vegetal hemisphere. When the cell bodies begin to elongate for cleavage, the polar fountain figures of the rays are also seen quite definitely. Let us see if these division features of an excentric egg can be harmonized with the concepts of the proposed theory.

In the first place it might be anticipated that the furrow would appear earlier at the side nearer the mitotic figure (the animal pole in *Astriclypeus*), because, during the initial stage of cleavage when the rays are crossing, for a given elongation of the spindle the tips of the shorter rays would be pulled in more than those of the longer rays. Later, after the crossing of the rays is dissociated, a wider gap would form between the parts of the asters with shorter rays than between those with longer rays, resulting in a deeper furrow in the region of the shorter rays. So far the concepts are quite adequate, although they were originated to explain the division of eggs with centrally located nuclei. Let us repeat them: The asters are spiny gel spheres and cleavage is the consequence of their separation by the elongation of the spindle.

There is, however, a contradiction. For if an excentric spindle simply elongates in the ordinary fashion, even though the furrow forms earlier on the side where the rays are shorter, by the time cleavage is nearly completed the blastomeres ought to assume the shape shown in Figure 4. Since this never happens in actual cleavage, some other factor must be operating.

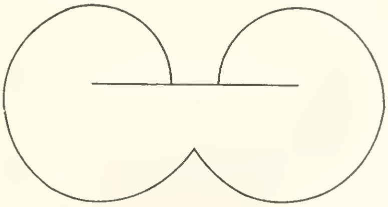

FIGURE 4. The probable shape of the blastomeres if an excentric spindle should simply elongate without bending.

Perhaps another model, this time one with the spindle and astral centers located above the middle of the egg, will help to explain this factor (Fig. 5, *A*). If the asters are pushed apart and their positions fixed by putting a pin through the holes in the bars representing the spindle, these automatically form an angle (Fig. 5, *B*). Since no special rotating force is being applied under this circumstance, the flexion of the "spindle" must be the direct result of the simple separation of the "asters." If this model experiment conveys a correct picture, it leads directly to the suggestion that the spindle may be bending during the cleavage of *Astriclypeus* eggs.

In the model, the mechanical force causing the flexion of the bars is very easy to understand. The tips of the rays (bamboo rods) are attached to the cortex (rubber band). Therefore, when the astral centers are pushed away from each other by a given distance, the tips of the rays are left behind by that distance in relation to the astral centers. Under such a circumstance, the shorter rays have to rotate through larger angles than the longer rays in order to cover the shifted distance. This difference between the rotating angles of the rays of the two sides will work, in the net result, so as to rotate the whole aster in the direction of the shorter rays, provided that the aster is rigid and can maintain a constant shape. In the model, these rotations naturally force the two bars, each attaching to one "aster," to make an angle between them.

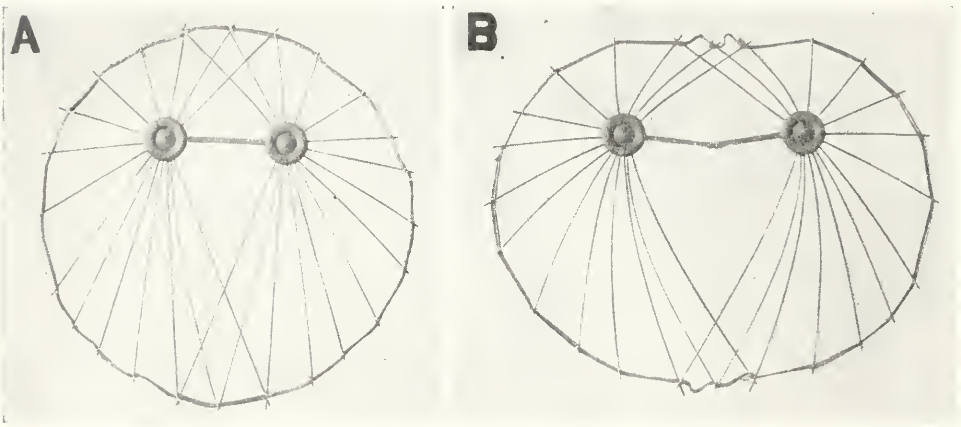

FIGURE 5. A model similar to that shown in Figure 3, except that the spindle and the astral centers are made excentric (*A*). In *B*, after separating the astral centers and fixing their positions by putting a pin through the holes in the bars, note the following points: (1) The fountain figure of the polar rays is obscured and the majority of the rays turn in the same direction in a vortex configuration (compare with Fig. 3*B*). (2) The innermost pair of the crossing rays at the upper pole is practically straight while that of the lower pole is clearly bending and finally (3) the spindle is flexed in the middle.

The model, however, still cannot imitate the expansion phase, because, being two-dimensional, it cannot take care of the factor of the constancy of the cell volume. But, *a priori*, since the furrow surface is sucked into the gap between the two asters in the expansion stage, the rotation effect will be more emphasized: viz., during the expansion phase of actual cell cleavage, the spindle will bend more acutely, to such an extent that it will even tend to tear open the vegetal region, forming the furrow. It must be pointed out here that in order to allow the free rotation of the two asters around their excentric centers, the volume of the cells would have to be increased, as can be judged from Figure 6. If the volume of the cell were kept constant, the rotation of absolutely rigid asters would be impossible. The fountain-figured bending of the polar rays is also quite significant in this connection, as it makes possible astral rotation within cytoplasm of a constant volume.

SPINDLE BENDING IN ASTRICLYPEUS EGGS

The next step was to determine whether the spindle is actually bending in the *Astriclypeus* eggs. Even a hasty examination was enough to confirm this fact.

On further investigation, it came to the authors' notice that though the degree of excentricity of the mitotic figure is fairly constant within a single batch of eggs, it varies somewhat among different batches, and the acuteness of the spindle bending is proportional to the degree of its excentricity. In speaking about the degree of excentricity, in order that the statement be accurate, the eggs must be observed strictly from the side.[1] Naturally, the cases with extremely excentric spindles are most convenient for analysis. In Figure 7 are given tracings of micro-photographs showing the mode of cleavage and the conditions of ray bending. Purposely, in the first and the last drawings of Figure 7, eggs with less excentric spindles are shown, while the second to the sixth are successive stages in the cleavage of a single egg with an extremely excentric spindle. In diagrammatization, however, the results of many other observations were taken into consideration.

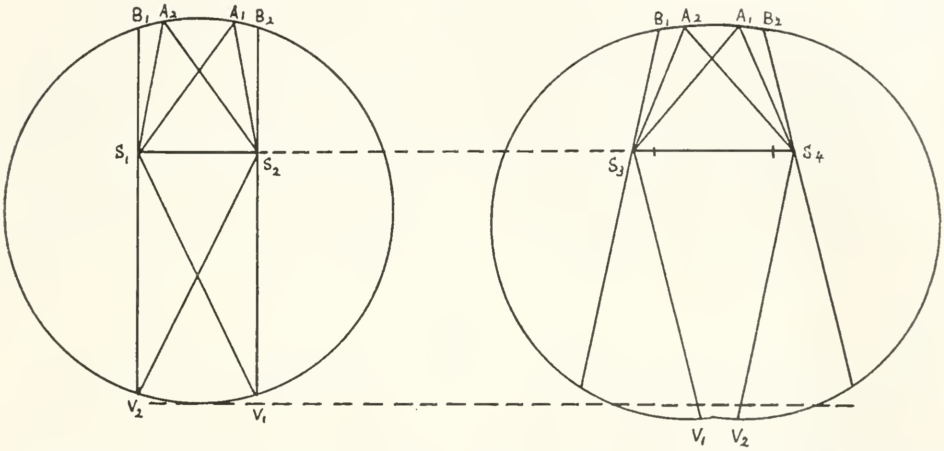

FIGURE 6. The hypothetical result of the simple rotation of asters around their excentric centers, as the result of the shrinkage of the upper pole. Notice the opening of the lower side accompanied by the stretching of the surface there and the bulging of the lower contour. In the actual cell division, such a perfectly free rotation is prevented because the cell volume is kept constant. But the rotation is allowed within a limit corresponding to the degree of bending of the polar rays.

In the first drawing, as far as could be judged from observation on living material and photographs, the astral rays and the spindle are both straight. (Remember that this is a case with less excentric spindle.) Theoretically, in this stage, the rotation of the aster must have begun, but this could not be ascertained, presumably because of its slightness. In the second drawing (an extreme case), the animal furrow is decidedly in the expansion phase. At this stage, two fan-shaped areas appear, one on each side of the cleavage furrow, in which the rays seem to be fewer than in other parts so that these regions are slightly more transparent and

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[1] In order to obtain a strict side view of an egg, one takes advantage of the fact that the spindle excentricity is exceedingly constant within one batch. On surveying a culture of eggs with a medium magnification, one can easily get the idea of the maximum excentricity of the spindle as far as that particular batch is concerned. Such maximal cases must represent a full side view of the egg.

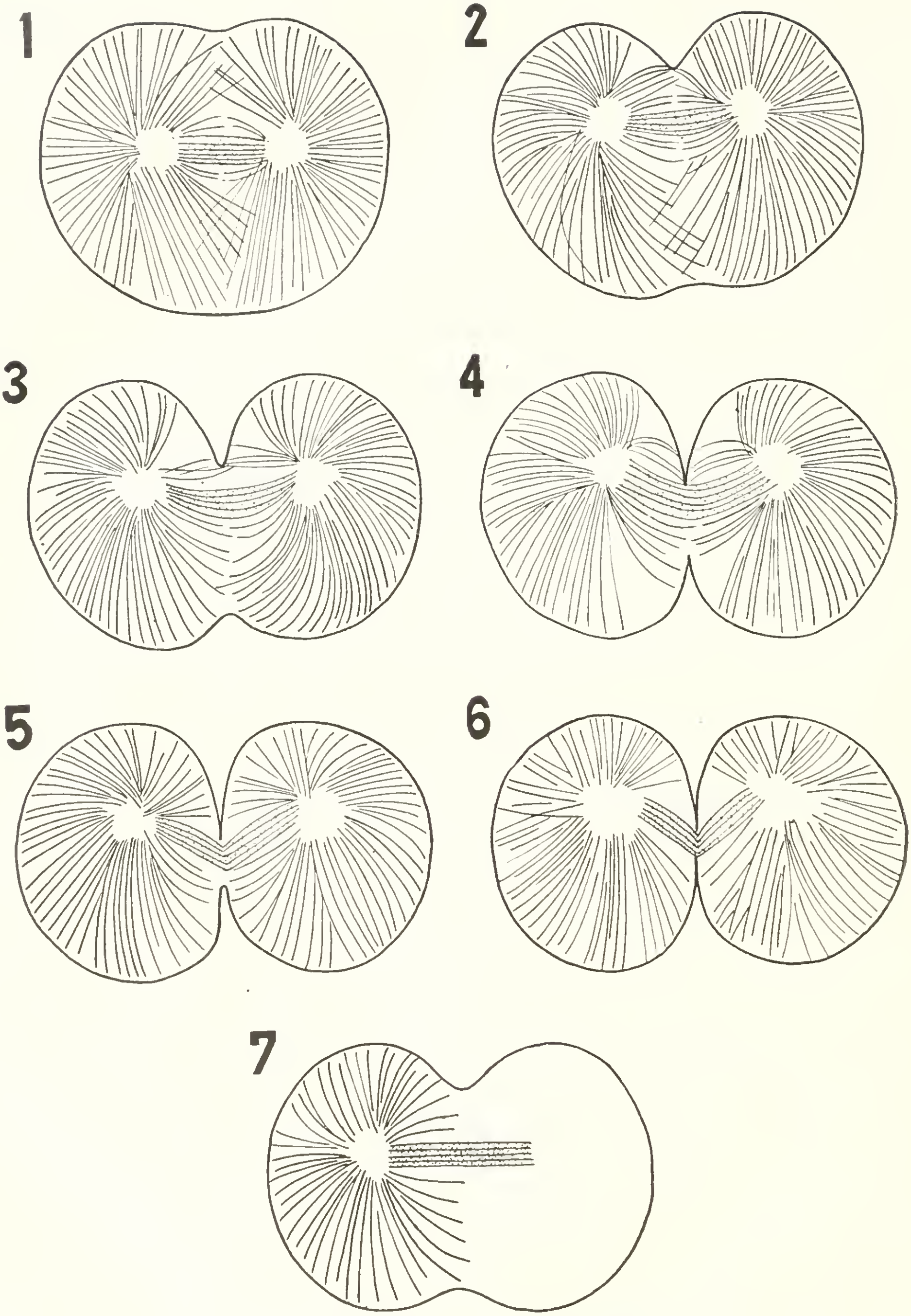

FIGURE 7. Tracings of micro-photographs of dividing *Astriclypeus* eggs showing the changes of curvature of the astral rays in successive stages. The first drawing is made from an egg of a different source but the second to the sixth drawings belong to a series of photographs of a

relatively bright. Since the authors were at first skeptical about this fact, special attention was paid to this point, but numerous observations only served to confirm it. According to the authors' interpretation, the appearance of the fan-shaped area is probably due to the fact that as the furrow begins to be drawn in by the suction effect, the formerly crossing rays are caught by the furrow constriction. Then in the fan-shaped portion just behind the furrow tip, the rays will become relatively sparse. This bundling of the equatorial rays by the furrow must certainly be the beginning of the formation of the so-called sheath rays, described by Yatsu in *Cerebratulus* eggs: "One striking feature of this stage is the formation of the sheath rays around the spindle, due to the fusion of the equatorial rays and a part of the intermediate rays. The spindle shaped sheath rays seem to occur in a good many forms" (Yatsu, 1909, p. 387). Simultaneously with the appearance of the fan-shaped area, the bending of the spindle becomes noticeable.

At the same time, two other changes are taking place in the curvature of the astral rays. One is the bending of the vegetal crossing rays. Among regularly dividing cells, the rays are straight while they are crossing, for they are supposed to be exerting traction force. Therefore, the bending of these vegetal crossing rays deserves especial attention (see Fig. 5). The other change is the curvature of the rays lying in the sector between the fan-shaped area and the spindle polar region. These rays, which have been either straight or bending in a fountain figure (see the right blastomere of the second drawing), now start to bend in unison into the same direction as the vegetal crossing rays, so that the rays of the whole aster display a beautiful vortical configuration (see the left blastomere of the second drawing). These changes in the way of bending of the rays can best be explained by thinking that the asters have been twisted around their centers. In the third drawing, the sheath ray formation on the animal side, the spindle bending and the vortex figure all advance in degree and the vegetal crossing rays have almost been drawn apart. This drawing is of particular interest in two senses. One is that it shows the vegetal half of the egg actually being torn apart by the twist. The other is that it almost coincides with a drawing of Conklin's of the egg of *Cynthia partita* (Conklin, 1905; Pl. VII, Fig. 100). In the fourth stage, the furrow on the animal side has almost reached the spindle and the sheath rays there begin to show a tendency to return to their original positions. On the vegetal side, two fan-shaped areas can be recognized, indicating that sheath ray formation has begun there also. Moreover, the formerly

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single egg. The spindle of this egg is more excentric than that of the first drawing. The seventh drawing is an example of an egg with a far less excentric spindle. Drawing 1, rays are practically straight. Theoretically the asters must have begun to rotate, but this cannot definitely be ascertained. Drawing 2, note the appearance of the fan-shaped clear areas on both sides of the animal furrow in the expansion phase; sheath ray formation; the bending of the vegetal crossing rays; the slight sign of spindle bending. Drawing 3, further development of the fan-shaped bright portion; advance in the spindle bending and dissociation of the crossing rays of the vegetal side. Note also the vortex configuration of the rays. Drawing 4, approximate contact of the animal furrow with the spindle and the tendency of the rays of the animal side to return to a straight condition. Appearance of the fan-shaped areas on the vegetal side. Drawing 5, last moment of division. Disappearance of the fan-shaped areas and definite bending of the spindle. Drawing 6, interkinetic stage. Bending of the vegetal rays in the opposite direction (divergent bending). Drawing 7, division of a cell with less excentric spindle. Notice usual fountain figure of the rays instead of the vortex configuration and apparent straightness of the spindle.

bent vegetal rays seem to have returned to a straight condition. But judging from the fact that the rays on the animal side still retain the same curvature as in the previous stage, this straightening of the vegetal rays may have been induced by the change in the shape of the aster itself.

In the fifth drawing, the cleavage is almost complete and all the rays, including the former sheath rays, are returning to a straight condition. At this stage, the spindle forms an obtuse angle with its apex directed toward the vegetal pole. The sixth drawing shows the egg in the interkinetic stage. Now the vegetal rays are turning in the opposite direction and new fan-shaped, ray-free areas appear adjacent to the now bent spindle. In Yatsu's last drawing, this kind of bending is seen in the rays of the animal side. This post-cleavage bending is what the senior author called "divergent bending" in the previous paper (Dan, K., 1943b), and it is considered to be an indication of the formation of the new surface (see also Dan and Dan, 1940).

In the seventh drawing, the condition within an egg whose spindle is not so excentric is illustrated. Here no vortex configuration is attained and the seemingly straight spindle and the usual fountain figure of the polar rays are seen as in *Cerebratulus* eggs. In this latter form, the spindle is not so excentric and its bending, consequently, becomes apparent only in much later stages, as can be judged by Yatsu's figure.

These observations on the eggs of *Astriclypeus* with extremely excentric spindles help to elucidate the intrinsic mechanism of spindle bending. It seems almost certain that the causative force of spindle bending is derived from the advancing animal furrow and transmitted by the sheath rays to the astral centers which, in turn, impart it to the spindle. But since the advance of the furrow is, itself, nothing but the result of the pushing apart of the two asters by the elongating spindle, an interesting cycle is seen. Thus it can be said that the causative force of spindle bending comes from the spindle's own elongation.

It must be emphasized that the fundamental situation is not at all different from that of typical cleavage. In both cases, sheath ray formation occurs. But in typical cleavage, since the inward pressure which produces sheath ray formation occurs at both the animal and vegetal sides simultaneously, the two effects cancel each other. In the heart-shaped cleavage, the pressure begins at the animal side and for a short time there is nothing to counteract it on the vegetal side. During this time the elongating spindle will encounter resistance on one side only, and will become concave toward that side.

Two other important facts are patent in these observations. One is that the connection between the aster and the spindle is an unexpectedly firm one. There is a striking correlation between the degree of astral rotation and the degree of spindle bending. If this junction were loose, the aster might rotate more, but the spindle could not be bent at all. The second fact is that the spindle must have a certain degree of rigidity, since it can be bent by applying forces at its two ends. The present data give further, and more decisive, support to the possibility postulated by K. Dan (1943a).

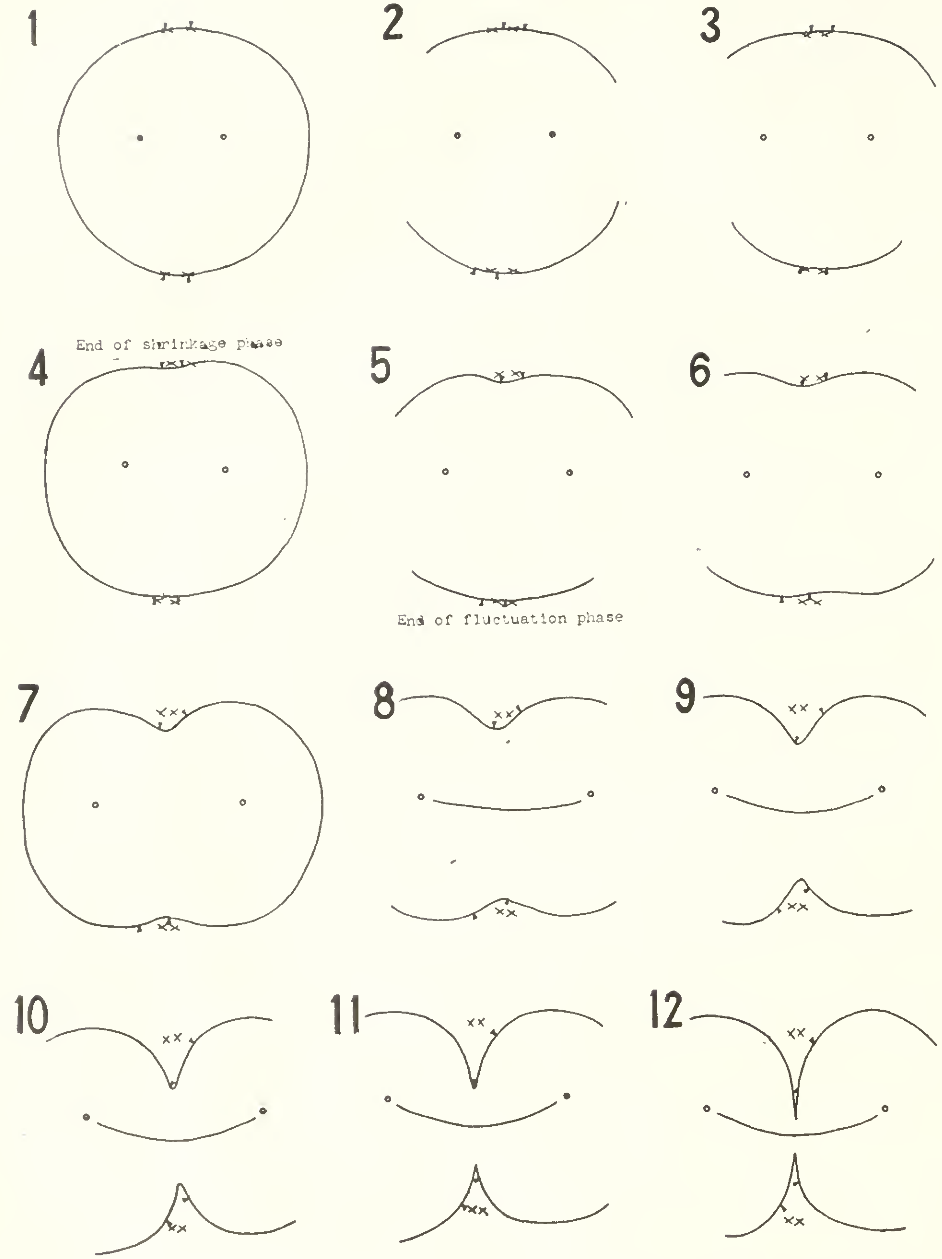
Lastly, it must be pointed out that although the model experiment helped to reveal the mechanism of spindle bending, the spindle bending during the shrinkage phase shown by the model could not be demonstrated within the living cell. In actual observation, the sheath ray formation is always seen as the direct impetus for spindle bending. Then is it permissible to apply this idea, obtained from a model, to actual cells?

The Effect of Astral Rotation on Surface Behavior

In the foregoing sections, it was argued from the model experiment that the cause of astral rotation lies in the difference in length of the astral rays on the two sides of an aster. If this is so, the rotation of the aster must be occurring from the very beginning of the cleavage process. Although the observations of the bending of the rays and of the spindle, such as were reported in the previous section, are quite decisive, they can be applied with certainty only to the expansion phase in which the sheath ray formation becomes evident.

For information on the early part of the cleavage process it was thought that a study of surface behavior might be used to establish some unmistakable evidence of astral rotation. Here the kaolin method and its geometrical analysis were employed again. The purpose of the analysis was to test whether or not the furrow formation is really due to the traction effect of the crossing rays. The procedure was to make a sketch of an egg in the spherical condition on which the positions of the kaolin particles and of the astral centers were recorded. From this sketch, the lengths of the crossing rays could be obtained. Once these lengths were fixed, given the spindle length of each stage, the theoretical positions of the particles could be obtained for later cleavage stages. These theoretical positions of the particles could then be compared with their observed positions. If the authors' postulates be correct two conditions follow: (1) The theoretical positions of the particles ought to fall on the sketched contour of the cleavage furrow because the surface is being pulled in by the rays during the shrinkage phase and (2) the degree of shrinkage in the distance between the two theoretical points ought to agree with that of the observed particle-distance as long as the particle-distance coincides with the real crossing range of the rays. In the previous paper of this series, it was shown that the above propositions satisfactorily hold in actual cases (Dan, K., 1943b; Figs. 6 and 7, Table III). But it must be stressed that even when the second condition fails, the first condition proves to be true as long as the deviation between the actual particle-distance and the crossing range is not too great. This means that the second proposition is a stricter test for the hypothesis than the first one. However, in order to apply the second proposition properly, the painstaking accumulation of many readings is necessary to map out beforehand the actual range of crossing of the rays (see Dan, K., 1943b; Fig. 8). On the contrary, the first proposition, although it is less sensitive, is convenient and quite satisfactory as a qualitative test. When the suction force develops and the crossing rays are pulled apart, the intersection points (apices of the triangles) begin to fall outside the contour of the cleavage furrow. The time at which the intersection points begin to deviate is always simultaneous with the onset of the expansion phase.

Now let us see how the surface of *Astriclypeus* eggs behaves in the kaolin experiment. The method has been used on these eggs previously (Dan, Dan and Yanagita, 1938) but the present observations are especially concerned with eggs containing extremely excentric nuclei. Two sets of the records for the qualitative test are given in Figures 8 and 9 and Table I. As for the animal furrow, in the egg shown in Figure 8, the expansion phase sets in at the fifth stage and at this very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End of shrinkage phase
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End of fluctuation phase
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FIGURE 8. Successive camera lucida drawings of a cleaving *Astriclypeus* egg with the recorded positions of kaolin particles (▼▼) and astral centers (○○). In the spherical condition (first drawing), the two particles at the animal pole and at the vegetal pole respectively are connected to the astral centers by four hypothetical rays (which, however, are omitted from the figures). In succeeding drawings, by using the sets of four lines obtained in the first drawing as radii and taking the astral centers of successive stages as centers, two intersection points

stage, the intersection points start to deviate from the contour. In the egg shown in Figure 9, this happens at the sixth stage and the coincidence between the onset of the expansion phase and the initiation of the deviation of the intersection points from the contour is again unfailing. As far as the vegetal furrow is concerned, in the egg of Figure 8, the particle-distance follows a zig-zag course: 100.0%–97.9%–111.4%–96.9%–104.1%. At the sixth stage, the value jumps to 121.8% which is followed by a further rise. Therefore, the sixth stage can be considered as the beginning of the expansion phase. In the egg of Figure 9, although the course of change is more gradual, the same tendency is unmistakably recognizable: 100.0%–104.0%–103.0%–104.3%–104.6%–100.0%–100.3%. At the eighth stage, the value goes up to 115.2% and the intersection points begin to fall outside the furrow contour. These examples indicate that in eggs with extremely excentric spindles, the initial shrinkage effect is so obscured at the vegetal side that the term "shrinkage phase" is no longer appropriate. For this reason, in the present paper, the term "fluctuation phase" will tentatively be used. But a rather surprising fact is that in Figure 8 the intersection points coincide well with the traced cell contour during this fluctuation phase. In Figure 9, the intersection points do not at least go outside the drawings until the end of the fluctuation phase. From these facts, it seems permissible to conclude that at both poles of the cleaving *Astriclypeus* eggs, fundamentally the same division mechanism is at work.

Then why is this expressed as shrinkage at the animal side and alternate shrinkage and expansion at the vegetal side? It may be due to the rotation of the asters. It was suggested in the foregoing section that when the two asters rotate so that their animal hemispheres converge, the vegetal furrow must tend to be torn open. In reality, the situation may be somewhat more complicated. While the asters turn, if the tips of the crossing rays can retain their attachment to the cortex, the vegetal surface may be made to shrink more than when the rotation is absent. But if these rays pull loose from the cortex and the asters become free to rotate, they may stretch the surface between them on rotation as is diagrammatically shown in Figure 6. If the rays pull loose in several steps shrinkage and expansion will alternate.

Another point which is rather peculiar is the shift of the intersection points to the inside of the cells in the drawings, a form of behavior which has never been met with in regularly dividing cells. For example, in Figure 9, during the fluctuation phase of the vegetal furrow which lasts for seven stages, the intersection points fall inside the cells at two different times including four stages. Stage 3 and stage 5 of Figure 9 are of special interest. In those two stages, the animal furrow being still in the shrinkage phase, the intersection points of that side are coinciding with the contour. As long as the intersection points of the animal side are coinciding with the contour, one is provided with a guarantee that no error is involved in the recording of the positions of the astral centers. Yet the vegetal intersection points fall inside the drawing. This shows that the inward shifting of the vegetal intersection points is not an error but represents something which is actually happening

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(××) are found for each pole. The percentage changes of the observed particle-distance (P) and of the theoretical distance between the two intersection points (D) are given in Table I together with the time of recording. Note that during the shrinkage phase of the animal pole (from the 1st to the 4th stage) and the fluctuation phase of the vegetal pole (from the 1st to the 5th stage), the intersection points fall on the egg contour.

in *Astriclypeus* eggs. There are two suggestions as to its identity. One is spindle bending. Since the bent spindle is convex toward the vegetal pole, it may push out the contour of the vegetal furrow. The other is that when the asters rotate around their excentric centers, pulling loose their crossing rays, the vegetal contour may bulge out as is indicated in Figure 6. At any rate, the astral rotation may

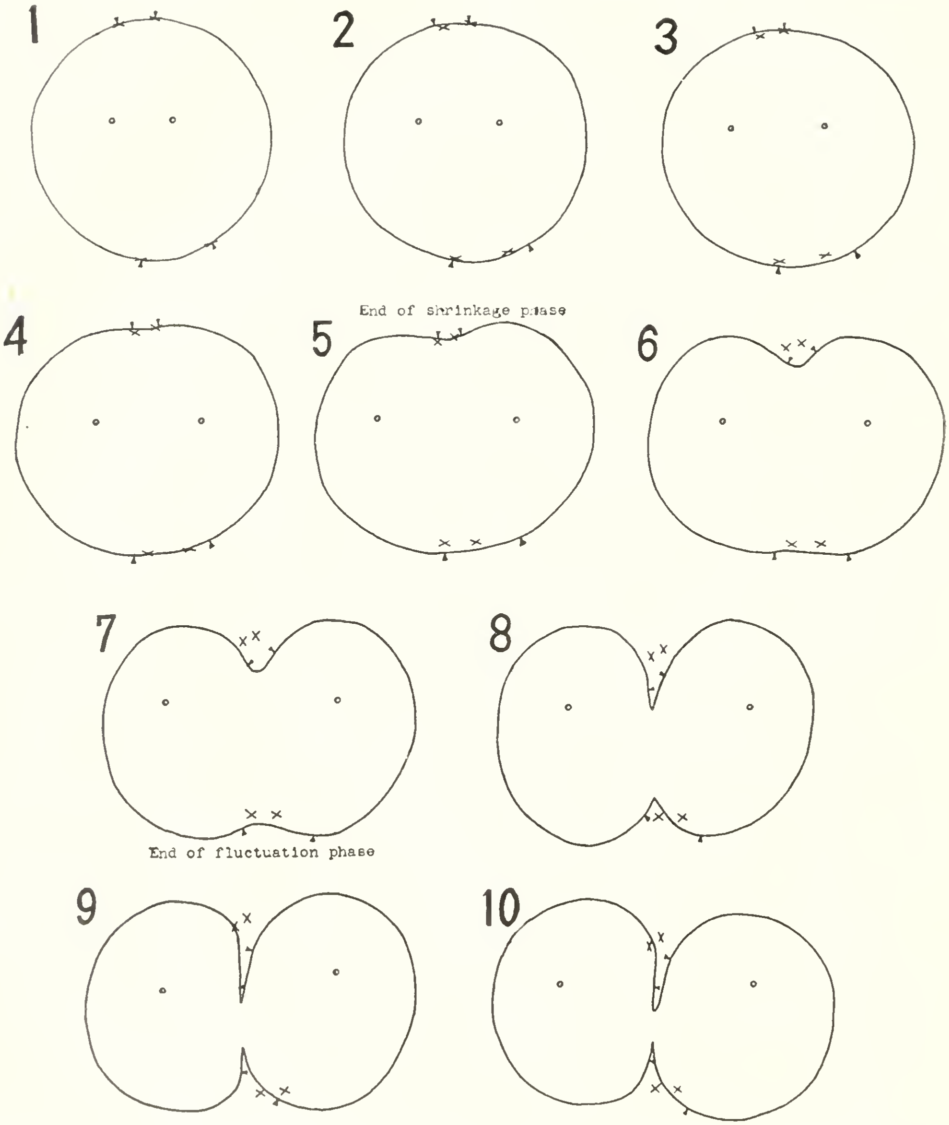


FIGURE 9. Another example of the geometrical analysis of an egg with extremely excentric spindle. The shrinkage stage of the animal pole lasts for five stages and the fluctuation of the vegetal pole ends at the seventh stage. Note that the vegetal intersection points are sometimes inside the contours.

TABLE I

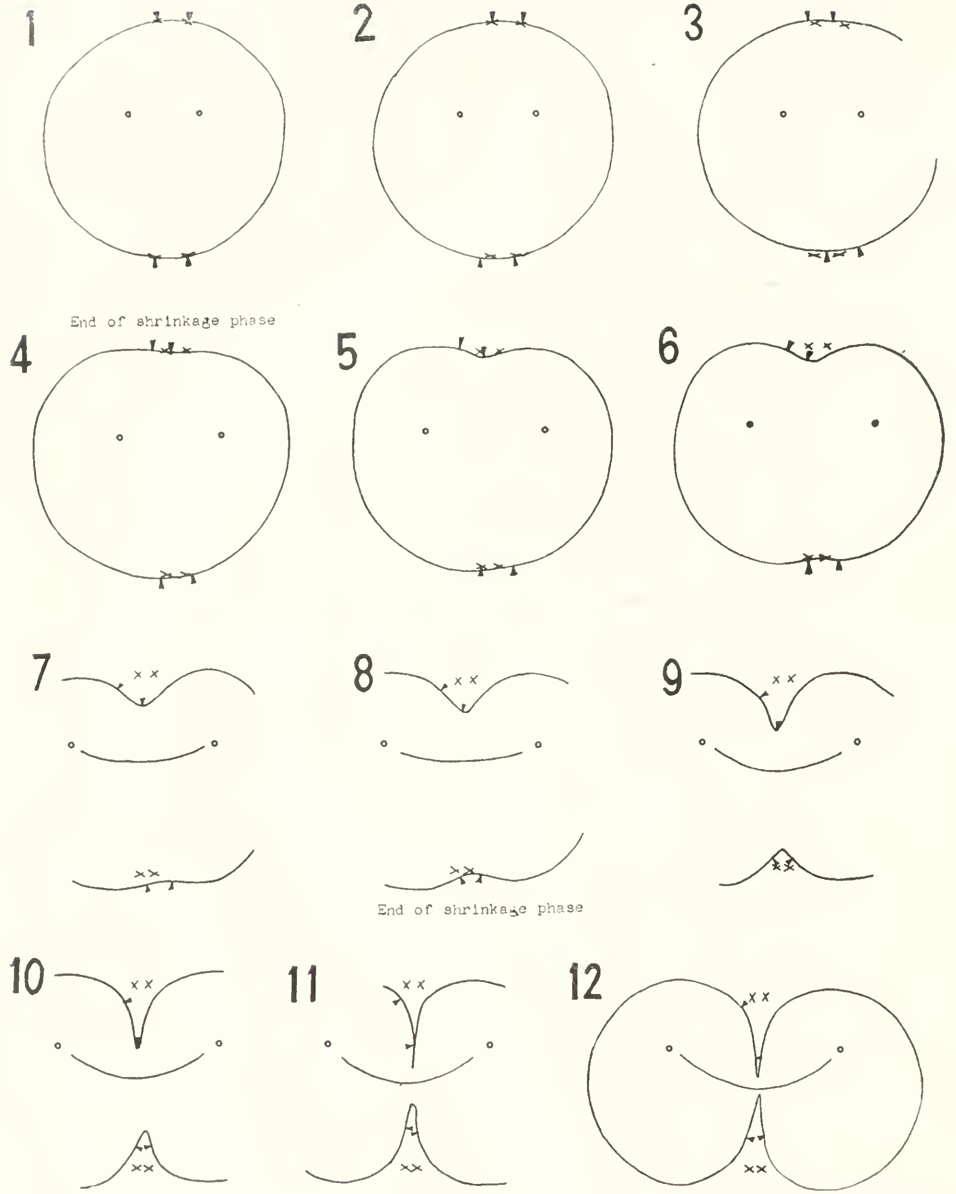
The results of the geometrical analyses of *Astriclypeus* eggs with extremely excentric spindle shown in Figures 8 and 9, exemplifying the "fluctuation phase." P, percentage changes of observed particle-distance. D, percentage changes of theoretical particle-distance.

The case of Figure 8

Stage	1	2	3	4	5	6	7	8	9	10	11	12
Time of recording at 21.0° C.	0″	1′00″	2′00″	3′00″	3′50″	4′40″	5′00″	5′45″	7′40″	8′38″	9′30″	10′35″
Animal furrow	$P=100.0$ $D=100.0$	$P=93.7$ $D=88.5$	$P=92.7$ $D=75.0$	$P=83.3$ $D=83.3$	$P=97.9$ $D=65.6$	$P=119.7$ $D=61.4$	— —	— —	— —	— —	— —	— —
Vegetal furrow	$P=100.0$ $D=100.0$	$P=97.9$ $D=91.6$	$P=111.4$ $D=90.6$	$P=96.9$ $D=85.4$	$P=104.1$(?) $D=69.7$	$P=121.8$ $D=63.5$	$P=127.0$ $D=61.4$	— —	— —	— —	— —	— —

The case of Figure 9

Stage	1	2	3	4	5	6	7	8	9	10
Time of recording at 26.5° C.	0″	2′00″	2′50″	4′05″	5′00″	7′00″	8′05″	9′30″	10′45″	12′30″
Animal furrow	$P=100.0$ $D=100.0$	$P=94.0$ $D=73.5$	$P=80.8$ $D=68.8$	$P=72.8$ $D=59.6$	$P=66.9$ $D=47.0$	$P=99.3$ $D=43.0$	$P=114.5$ $D=39.1$	$P=158.9$ $D=39.7$	$P=185.4$ $D=39.7$	$P=218.5$ $D=39.7$
Vegetal furrow	$P=100.0$ $D=100.0$	$P=104.0$ $D=73.3$	$P=103.0$ $D=63.0$	$P=104.3$ $D=55.4$	$P=104.6$ $D=45.5$	$P=100.0$ $D=39.3$	$P=100.3$(?) $D=33.0$	$P=115.2$ $D=31.7$	$P=122.1$ $D=33.0$	$P=123.8$ $D=30.4$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End of shrinkage phase
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End of shrinkage phase
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FIGURE 10. Geometrical analysis of an egg with a less excentric spindle. Both the animal and the vegetal poles undergo a shrinkage. The vegetal intersection points are inside the drawings at the seventh and the eighth stages with bent spindles.

TABLE II

Data of the geometrical analysis of an *Astriclypeus* egg with a less excentric spindle (see Fig. 10). Designations same as Table I.

Stage	1	2	3	4	5	6	7	8	9	10	11	12
Time of recording at 22.5° C.	0″	1′00″	2′15″	2′30″	3′15″	4′00″	5′00″	6′40″	7′35″	8′35″	10′00″	11′35′
Animal furrow	$P=100.0$ $D=100.0$	$P=90.6$ $D=88.2$	$P=78.1$ $D=88.2$	$P=58.5$ $D=66.4$	$P=85.1$ $D=54.6$	$P=75.0$ $D=56.2$	$P=107.8$ $D=46.8$	— —	— —	— —	— —	— —
Vegetal furrow	$P=100.0$ $D=100.0$	$P=100.0$ $D=97.7$	$P=98.4$ $D=88.7$	$P=93.2$ $D=73.1$	$P=97.7$ $D=62.4$	$P=91.7$ $D=63.9$	$P=83.4$ $D=50.3$	$P=81.9$ $D=50.3$	$P=84.2$ $D=45.8$	$P=121.0$ $D=45.1$	— —	— —

offer the best single explanation for both the fluctuation phase and the inward shift of the vegetal intersection points.

In the second paper of this series, the authors (Dan, Dan and Yanagita, 1938) handled the data of the kaolin experiments on the eggs of this sand-dollar as if there was no qualitative difference between the behaviors of the two poles of the cell. Even though they are sure that this omission did not affect the conclusion of that paper materially since they were not limiting themselves to the extremely excentric cases, they wish to make this point clear.

Finally, the eggs with less excentric spindles must be examined. In one sense, this test is very important because in eggs with less excentric spindles, their bending can be noticed only in a very late stage of the cleavage process. The data are given in Figure 10 and Table II. As can be seen from the table, the particle-distance on the animal furrow reaches its minimum at the fourth stage. Until this time, the intersection points remain on the contour. The vegetal furrow, in this case, undergoes a shrinkage similar to other examples reported in the previous paper. In this particular case, the vegetal furrow remained in the shrinkage phase for an exceptionally long time, i.e., until the eighth stage. Yet the intersection points stay on the contour through this period, except the seventh and the eighth stages where the vegetal intersection points are inside the contours concurrent with the onset of spindle bending. On closer examination of Table II, however, it will be noticed that the degree of shrinkage is much less for the vegetal furrow (minimum—81.9%) than for the animal furrow (minimum—58.5%). Is this, then, due to the pulling loose of vegetal rays as a result of astral rotation? Here, the stricter test—i.e., the numerical fit between the theoretical and observed degrees of shrinkage—must be resorted to. It was pointed out that these figures coincide only when the positions of the kaolin particles fall exactly at the tips of the crossing rays. This occurs purely by chance. But if numerous records are examined, such cases will inevitably be met with. This is true with the animal pole of the *Astriclypeus* eggs as shown in Table III and Figure 11.

On the contrary, for the vegetal furrow, no case of an exact fit has been found in the entire mass of records. In every case, the degree of the observed shrinkage was far less than that of the theoretical shrinkage, no matter how small a particle-distance was chosen. (When a particle-distance is wider than the actual crossing range, the observed degree of shrinkage becomes less than that of the theoretical shrinkage (consult Figure 2).) The case given in Figure 10 and Table II well exemplifies the situation. This is decisive enough evidence that the asters must

TABLE III

An example of a nearly perfect fit between the observed (P) and the theoretical (D) degrees of shrinkage at the animal furrow of the *Astriclypeus* egg (see Fig. 11).

Stage	1	2	3	4	5	6
Time of recording	0″	2′05″	3′10″	4′00″	6′25″	6′55″
Observed shrinkage Theoretical shrinkage	$P=100.0$ $D=100.0$	$P=93.0$ $D=96.5$	$P=89.5$ $D=88.3$	$P=83.7$ $D=81.3$	$P=73.2$ $D=69.8$	$P=131.3$ $D=58.1$

be rotating also in the eggs with less excentric spindles where the early detection of spindle bending is difficult.

In short, the above result indicates that in the early stages of cleavage of the cases of less excentric spindles, the asters are rotating although spindle bending is not detectable. This may mean that while, as was pointed out above, the connection between the spindle tip and the astral center is surprisingly firm, it is not absolutely rigid and a certain degree of astral rotation may be induced by the pressure of the furrow on the sheath rays before the spindle is noticeably affected.

Spindle Bending in Other Forms

The bending of the spindle in *Astriclypeus* has been discussed and is shown diagrammatically in Figure 7. Other observations of spindle bending are not scanty in the literature. Yatsu's last drawing clearly illustrates it for *Cerebratulus*. Among other egg cells, Conklin (1902) shows many drawings of spindle bending in *Crepidula* eggs (Plate III, Figs. 60, 61; Plate V, Figs. 80, 81), Kuhn (1913) reported it in the summer eggs of *Polyphemus* (Fig. 15) and Rhumbler (1901) in the eggs of *Rhabdonema* (?). From both the drawings and the original texts of these reports, it is evident that the spindles lie excentrically in all cases.

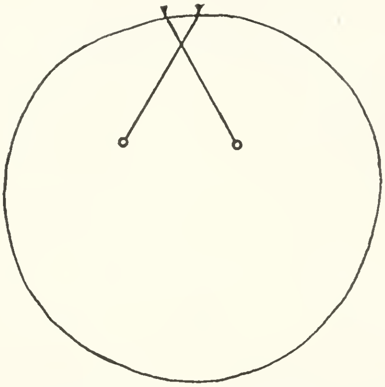

Figure 11. Sketch of the egg, the data of which are given in Table III, showing the range of crossing of the rays at the animal pole.

Examples of spindle bending are also found among male sex cells. Wilson takes up this subject in his book (1925, p. 140) in connection with the division of the spermatogonia of the grasshopper, *Rhomaleum*. Among recent workers, Sato (1940) touches this point in the spermatogonia (Figs. 18, 48) and the spermatocytes (Figs. 20, 50) of the scorpion, *Buthus martensii*, and he discusses it as a possible cause of the rosette arrangement of these cells within their cysts. He kindly informed the authors in a personal communication that, in these cases, the position of the resting nucleus and subsequently of the spindle is excentric. He also asserted that the bending of the spindle is not due to a lack of space within the cyst. From the above references, the authors believe there is enough evidence to support the proposition that spindles bend whenever they occur excentrically in cell bodies. As a matter of fact, once aware of this situation, careful observation can frequently catch slight bending of the spindle even in the so-called "regularly dividing" sea-urchin eggs, in batches in which the spindle lies more or less off the geometrical center of the cell (*see* Fry, 1937; Figs. 1–5). Another fact of special importance is that some of the investigations cited above include figures of spindle bending which occur well before a cleavage furrow is formed, thus definitely eliminating the possibility that the spindle is passively bent inward by the advancing cleavage furrow.

Artificial Induction of Spindle Bending

The foregoing analysis furnishes evidence that the cause of spindle bending is an unbalanced condition of the mechanical strains between the animal and vegetal sides of the spindle. If such is the case, by experimentally causing a similar unbalanced condition of force, it should be possible to induce spindle bending in a material in which it is not seen normally. This is achieved by the use of ether. The eggs of any kind of echinoid which normally divide in a symmetrical fashion can be used. The eggs are fertilized and a short time before the first cleavage is due, the surrounding sea-water is changed to an ether-sea-water mixture (0.6% by volume). In ether, even though the nuclear division takes place, the cytoplasmic division is suppressed and binucleated cells are obtained (Wilson, 1901). If these eggs are later washed free from ether, at the second cleavage, two spindles are formed side by side. Under this circumstance, the cleavage furrow appears in a plane at right angles to these spindles and two binucleate cells result. As the furrow constricts the cell, each of the two parallel spindles will obviously be cut by the furrow only on its peripheral side. In the living condition, during the cleavage, two obtusely V-shaped spindles can be seen which, at the final stage, are held in contact at their apices, giving the form of an X. Sections of ether-treated *Strongylocentrotus* eggs at the second division are shown in Figure 12.

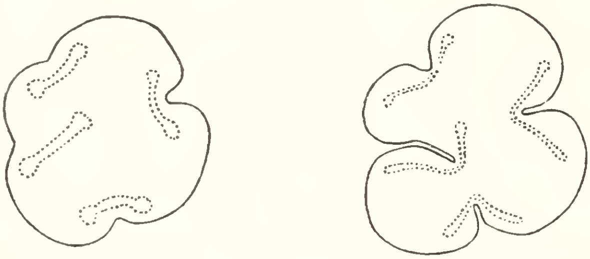

FIGURE 13. Reproduction of Yatsu's Figures 22 and 23 of a *Cerebratulus* egg, whose previous mitoses were inhibited by compression, about to cleave into four binucleate cells. The bending of the spindle is clearly shown.

In the literature, Boveri (1910) sketched a bent spindle in an *Ascaris* egg, the cleavage furrow of which was suppressed on one side by centrifugation. Yatsu (1908), in his experiments on *Cerebratulus* eggs with a compressorium, shows clear figures of spindle bending in an egg which is dividing simultaneously into four binucleate cells, even though his interest in that paper is on other points. Two of his figures are shown in Figure 13. This example suggests that whenever a cell divides simultaneously into many cells, spindle bending must be involved.

Discussion

In the foregoing sections, the authors have tried to analyse the cause of the bending of excentric spindles, and they have attributed it to the unbalanced mechanical resistance on the two sides of an elongating spindle resulting from its ex-

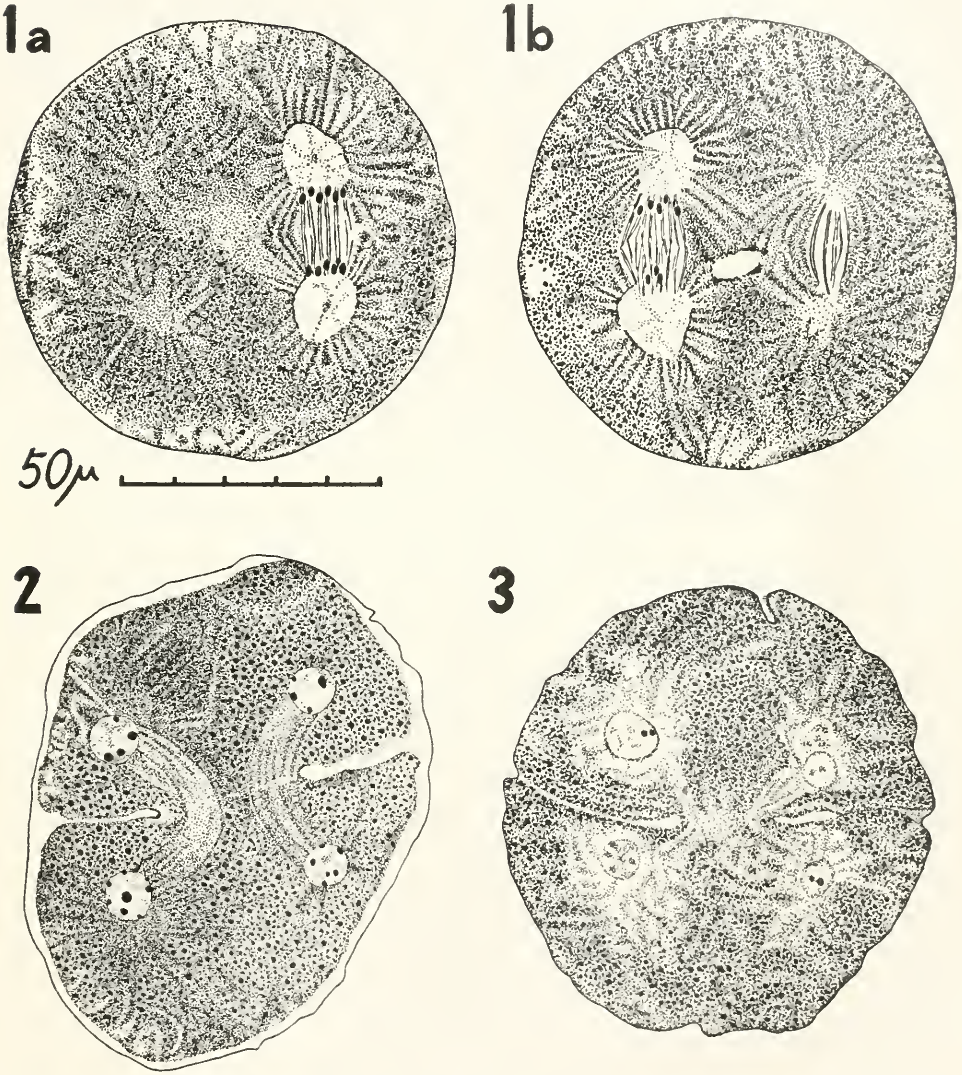

Figure 12. Sections of an egg of *Strongylocentrotus pulcherrimus* in the second division, the first cleavage of which was suppressed by ether. Fixed in chrome-formol mixture, 1*a* and 1*b* are two successive sections of an egg showing two spindles and four asters (the slit in the middle of 1*b* is an artefact). Two is an egg cleaving into two binucleate cells with the bending of the spindles. Three is another egg in a slightly later stage with sharply bent spindles, the spindles being much more vague in this case.

centricity. As far as the authors are aware, Gray is the only investigator who has tried to derive an explanation of this mode of division from a general theory on the division mechanism of regularly dividing cells. In discussing "astral cleavage" (Gray, 1931, pp. 201–202), he writes: "If the line joining the center of the two asters does not pass through a diameter of the egg by the time the astral rays reach one side of the egg, then the cleavage furrow develops first on that side, and only later (as the rays reach the opposite side of the equator) does the furrow develop on the other side. . . . That the irregularity of form and position of the asters is the cause and not the result of irregular cleavage is suggested by the fact that similarly irregular cleavages can be induced to occur in *Echinus* eggs by experimental means."

Although the authors readily support the latter part of his statement, they cannot help thinking that the mechanism he offers as the explanation for the formation of one-sided furrows is quite unsatisfactory. In the first place, his theory requires the existence of a stage in which the rays are reaching the nearer periphery but not the more distant side. This is quite contrary to the observed facts. It is obvious that the furrow formation starts with the elongation of the cell body only after the astral rays have filled up the cell interior. In the second place, even allowing the first requirement of Gray's theory, the way in which the furrow appeared on the nearer side by his mechanism would not be the formation of a furrow in the real sense of the words but would rather be the bulging out of the two sides of the furrow. Consequently, at the completion of such cleavage, the egg should have made headway toward the animal side, causing the spindle to take a central position. This is utterly incompatible with the observed facts.

A striking similarity between *Astriclypeus* and *Cynthia* eggs concerning spindle bending and the vortex configuration of the astral rays has been mentioned. However, the real situation seems to be much more complicated. Conklin (1905) states that the cleavage furrow of the 1st division starts from the vegetal pole near which the clear protoplasm and the mitotic apparatus are lying excentrically (*see* Conklin, 1905; Pl. II, Fig. 20). So far nothing contradicts the authors' expectations. But he further says that when the spindle begins to bend, toward the end of this cleavage, it becomes convex toward the vegetal pole from whence the furrow first appeared (Pl. VII, Fig. 100). He moreover asserts that this is also true for the first cleavage of *Ciona intestinalis*, (Pl. XI, Fig. 198). The direction of this bending is quite opposite to the one which can be expected from our theory.

On the other hand, for the second and third cleavages, the direction of the spindle bending is exactly what we would anticipate. He writes: "In the second cleavage the constriction of the cell begins at the periphery or free surface and proceeds inward through the cell body (figs. 104, 105). . . . and the middle of each spindle is bent in toward the center of the egg (fig. 105)" (p. 44). On page 46, he has another sentence to the same effect in connection with the first cleavage and with Figures 106, 107. A reexamination of ascidian egg cleavage is urgently needed.

From what has been discussed concerning the cause of spindle bending, it might be concluded that spindle bending is impossible in ordinary plant cells in which neither astral rays nor cleavage furrows are found. In spite of this, there are cases in which spindle bending has been reported among plant cells. Professors Sinotô and Wada of the Botany Institute kindly informed the authors of two such cases. One is in the pollen mother cells of a haploid *Triticum* reported by Gaines and Aase (1926) and the other is in the pollen mother cells of hybrid offspring of

Triticum and *Aegilops* studied by Kihara and Lilienfeld (1932). The cause of such bending may be quite different from that among animal cells and it must probably be sought for in the intrinsic make-up of the spindle itself.

The authors are happy to express their gratitude to Dr. Laura Hunter Colwin for her help in preparing this and the following manuscript, and to Dr. D. P. Costello and Dr. Daniel Mazia for their criticism and advice.

Summary

1. Study was made of the cleavage of cells with excentric mitotic figures.

2. In the division of such cells, (a) the cleavage furrow appears from the side of the cells nearest to the mitotic figure, (b) the asters rotate, causing the animal hemispheres to converge, and (c) the spindle bends, with the convex side directed toward the vegetal pole.

3. This situation can be explained by thinking that the mechanical resistance for the elongating spindle is different in magnitude on its two sides as a result of the excentricity of the mitotic figure.

4. The above situations can be artificially induced in materials which never show them normally, by producing a similar unbalanced resistance on the two sides of the spindle.

5. Gray's theory concerning the mechanism of the formation of one-sided cleavage furrows is discussed.

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BEHAVIOR OF THE CELL SURFACE DURING CLEAVAGE. VIII. ON THE CLEAVAGE OF MEDUSAN EGGS *

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Coelenterate eggs cleave characteristically with a one-sided advance of the furrow on the animal side, in contrast to utter quiescence on the vegetal side.[1] Around the beginning of the present century various theories were proposed to account for the mechanism of such division but they were highly hypothetical in nature, and failed to acquire much popularity. The present study, although admittedly preliminary in nature, is an attempt to clarify some aspects of the division of medusan eggs.

In previous papers the authors have postulated that the primary cause of cleavage is spindle elongation, both in regularly cleaving eggs such as those of the sea-urchin (Dan, 1943b) and also in cells with excentric nuclei (Dan and Dan, 1947). But in the asymmetrical furrow formation of eggs of the latter type, for example in the heart-shaped cleavage of *Astriclypeus manni,* there are secondary peculiarities not found in regular cleavage. The first is rotation of the asters about their centers, so that their respective animal hemispheres converge. This results from asymmetrical sheath ray formation, and can be detected by noting the change in direction of bending of the rays in successive stages of cleavage (Dan and Dan, 1947). The second peculiarity is the bending of the spindle, so that it becomes convex toward the vegetal pole. This is a direct result of the astral turning. Of course, such bending is possible only because the spindle itself is somewhat rigid, and the connections between the spindle tips and astral centers are firm.

Now, among different batches of *Astriclypeus* eggs, the excentricity of the spindle varies and the degree of one-sidedness of the furrow varies correspondingly. An orderly arrangement of these eggs, from less to more excentric spindles, will form a series continuous with the mode of cleavage of medusan eggs (Fig. 1). This might mean that medusan cleavage simply represents an extreme case of heart-shaped cleavage. If so, astral rotation and spindle bending should also be found in medusan eggs. The following investigation will test these propositions. Moreover, a few points specific to the present material will be presented.

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[1] Opinions differ as to which side of the medusan egg should be considered the animal pole. Ordinarily, the side at which the polar body is extruded and the cleavage furrow begins is called the animal pole. Schleip (1929) and Korschelt (1936), however, have adopted the opposite terminology. This is because the egg axis and the larval axis run in opposite directions among the Ctenophora. In order to avoid confusion, the terms "polar body pole" and "micromere pole" are sometimes used. In this paper, the term "animal pole" denotes the polar body pole and the term "vegetal pole" refers to the micromere pole, thus preserving a unity of expression throughout this series of papers.

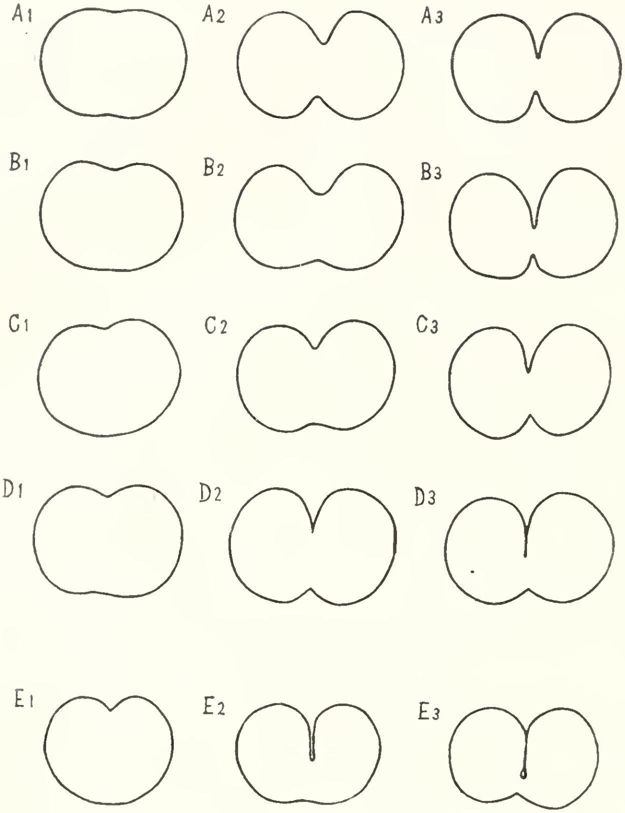

FIGURE 1. Comparison of the cleavage modi of sand-dollar eggs (*Astriclypeus manni*) and medusan eggs (*Spirocodon saltatrix*). *A–D,* cleavage of sand-dollar eggs arranged in order from less excentricity to more excentricity of the mitotic figure. *E,* cleavage of the medusan egg. Note that these make a continuous series.

The eggs of *Spirocodon saltatrix,* one of the *Tubularioanthomedusae,* are used throughout this work This medusa is quite abundant in the bays around the Misaki Station during the winter. The animals spawn on being put in the dark

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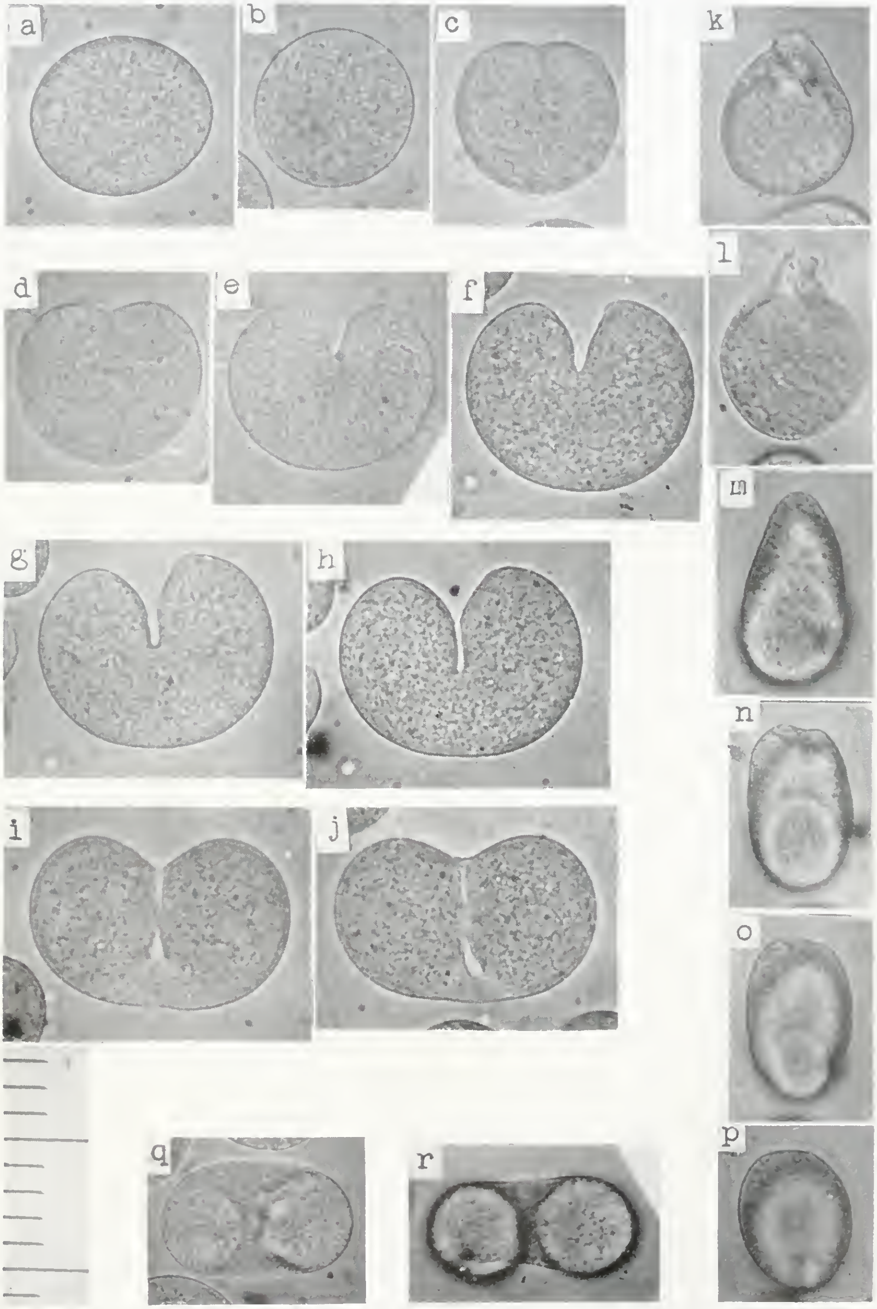
PLATE I. (*a*) Short time after fertilization. The egg pronucleus is vaguely visible at the extreme left side of the cell, which is the animal pole. (*b*) Conjugating pronuclei and the resulting mitotic figure are invisible by the usual microscopic observation. (*c*) and (*d*) Beginning of furrow formation. (*e*) Slightly more advanced stage. (*f*)–(*j*) Successive stages of cleavage of the same egg. (*f*) The spinning is not yet evident. (By this time, the fine protoplasmic processes must be coming out from the furrow surface; these are not visible unless observed under oil immersion.) (*g*) The amalgamation of the protoplasmic processes is partly completed. (*h*) (*i*) (*j*) The amalgamation products spread between the blastomeres in a sheet, fastening the cells together. (*k*)–(*p*) Views along the spindle axis. (*k*) (*l*) Initial stages of cleavage which roughly correspond to stage (*c*) of the side view. (*m*) More advanced stage. Note that the still uncleaved part between the blastomeres can be vaguely seen as a bright circle. (*n*) (*o*) (*p*) Successive stages of cleavage of a single egg. Notice that as the connecting bridge narrows down, the blastomeres round up. (*q*) View from above (animal pole). (*r*) View from below (vegetal pole). The smallest division of the scale is 10μ.

Plate 1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(Uchida, 1927). The reaction is particularly clear-cut if the animals have previously been exposed to electric light. Around 10° C., the first cleavage takes place from $1\frac{1}{2}$ to 2 hours after putting them in the dark, depending upon the latent period for spawning.

These eggs have absolutely no enveloping membrane in either the unfertilized or fertilized condition, as far as can be judged by microscopical observation. The egg pronucleus is clearly visible in the unfertilized condition and vaguely so during a short time after fertilization, taking an extremely excentric position within the egg (Plate I*a*). But by the time syngamy approaches, it is lost from view (Plate I*b*). When mitosis begins, the asters are discernible, although the rays are very faint and require oil immersion to be definitely distinguished (Plate II). For this reason, sectioning is necessary for detailed observation of the internal structures. When unfertilized eggs of *Spirocodon* are fixed in a chrome-formol mixture, the internal cytoplasm remains fairly homogeneous throughout the cell (Fig. 2; *1*). But if eggs are fixed by the same mixture after fertilization, the peripheral cytoplasm assumes a coarsely vacuolar aspect (Fig. 2; *2, 3, 4, 5*). On the other hand, if fixed by osmium, the cytoplasm of fertilized eggs is homogeneous (Fig. 3). From the above facts, it can be assumed that the peripheral vacuoles of the chrome-formol-fixed eggs are artifacts. If fertilized eggs are over-stained by neutral red or by Nile blue sulphate, many large heavily stained vacuoles appear in the peripheral zone of the cells. Such over-stained cells have already lost the capacity to cleave.

NORMAL CLEAVAGE OF THE EGGS OF SPIROCODON

Since the asters of this egg cannot be seen with certainty unless oil immersion is used, it is rather difficult to anticipate the exact time of the onset of cleavage. Most observations, therefore, were started after a slight indication of furrow formation had been noted. This drawback was somewhat offset by the slowness of the advance of the furrow.

When the furrow deepens, the egg tends to come to lie on its side, with the egg axis parallel to the substratum. As for the changes in contour in the side views of these cells during cleavage, there is not much to add to what has been reported for other forms (Plate I, *b* to *j*). If viewed from other angles however, an interesting feature appears. Photographs of two views perpendicular to the side view and to each other are shown in Plate I, *k* to *r*. From these, it is clear that cleaving *Spirocodon* eggs form flat plates of squarish shape. This is the reason why they tend to come to lie on their sides. Although sea-urchin eggs elongate in the direc-

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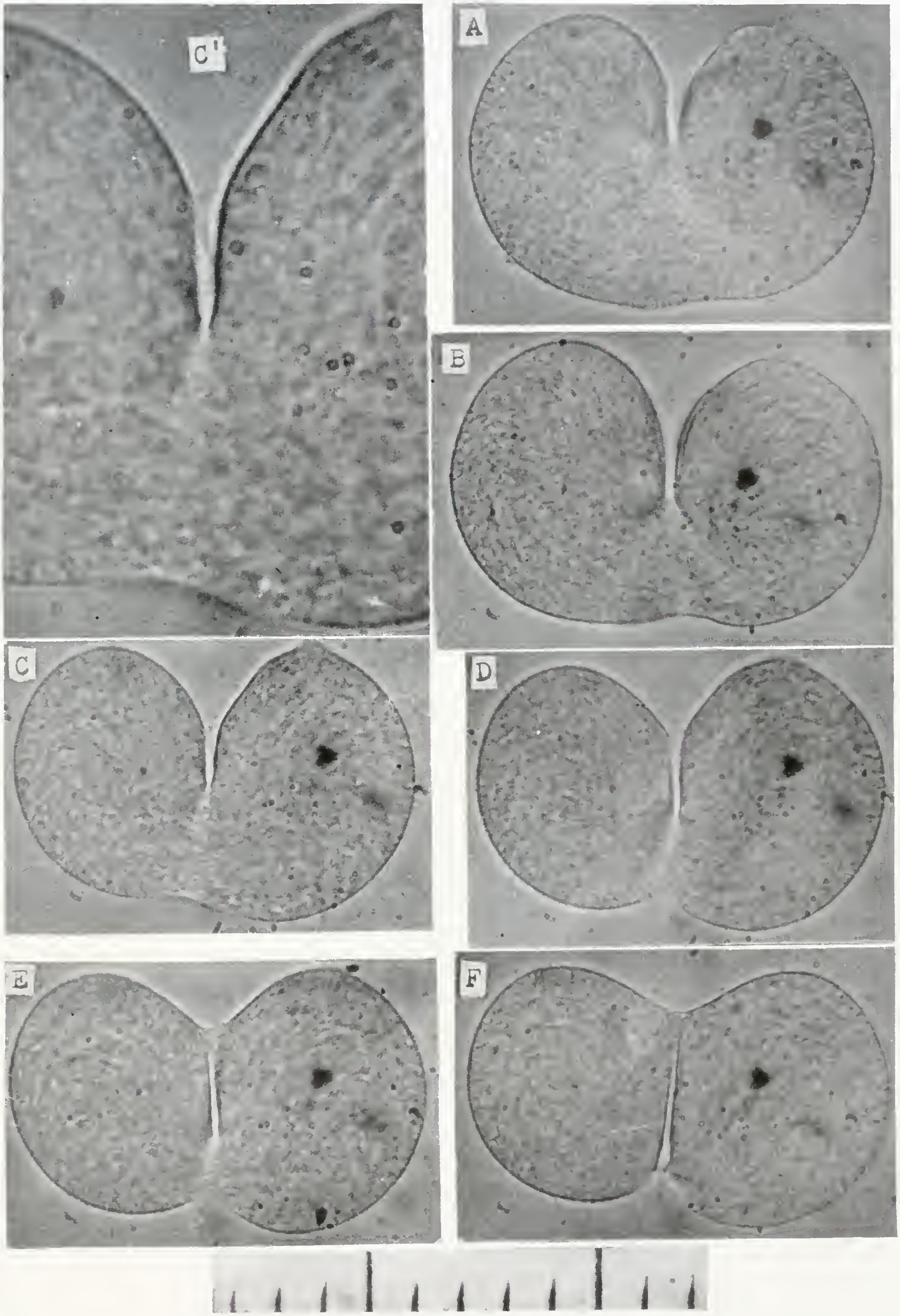
PLATE II. Successive stages of cleavage of a single *Spirocodon* egg observed with oil immersion lens. (*A*) Slight indication of the vortex figure within the blastomeres. No sign of the spinning activity within the furrow. (*B*) The vortex configuration is becoming more evident. The spinning activity has not begun yet. (*C*) The vortex figure is at its climax. The amalgamation of the protoplasmic processes is proceeding (*see C′*). (*C′*) Enlargement of *C* showing the spinning activity in the furrow. Note a longish shadow along the furrow space and several very faint lines running across the furrow, nearer the tip of the furrow. The latter are threads formed by the fusion of several protoplasmic processes which cannot be shown in the photograph. The former is the amalgamation product of the latter. This grows by further amalgamation into a sheet. (*D*) The spinning is complete around the entrance of the furrow. (*E*) Astral rays (vortex figure) are becoming vaguer. (*F*) The furrow has almost reached the vegetal side. The smallest division of the scale is 10μ.

PLATE II



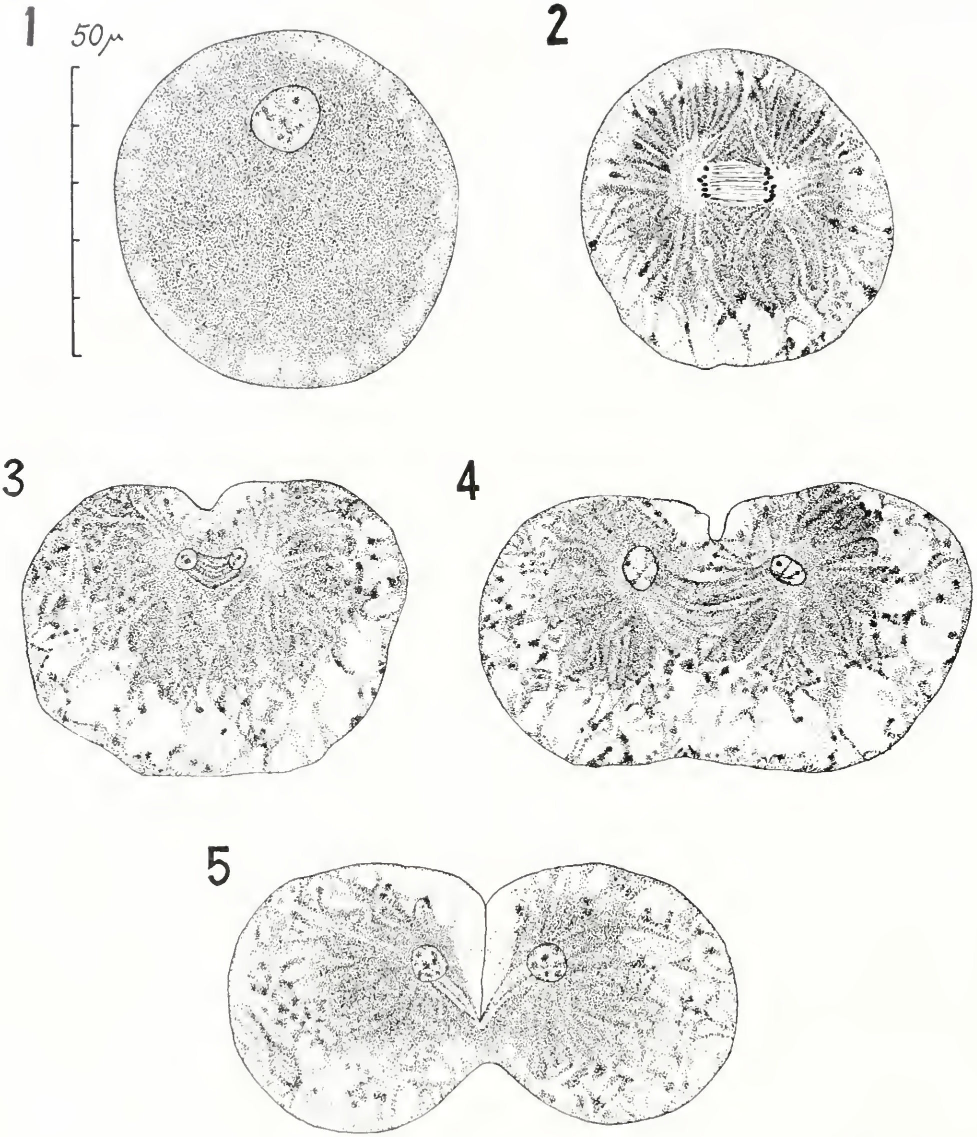


FIGURE 2. *Spirocodon* eggs fixed in chrome-formol mixture. *1*, unfertilized egg. The cytoplasm is fairly homogeneous with only a slight tendency to vacuolization around the periphery. *2*, telophase with chromosomes and asters. Note an intense vacuolization around the periphery. The plane of section is quasiequatorial; the peripheral vacuoles are seen encircling the island of dense cytoplasm. *3*, vertical section. Anaphase with asters, a bending spindle and a shallow furrow. Notice that the island of dense cytoplasm is definitely shifted toward the animal pole. *4*, a still later stage. *5*, a much later stage with a V-shaped spindle. Note that the vegetal vacuoles have now come around to the former animal side.

tion of the spindle, the shape of cross-sections parallel to the cleavage plane is always circular. Similar cross-sections of medusan eggs are a series of oblongs (Plate I, *m, n, o*), and only in later stages do the flattened incipient blastomeres gradually round themselves up (Plate I, *o* and *p*). This flattening is an important point to keep in mind for an understanding of the division mechanics of coelenterate eggs.

Several other things are notable in the side view of the egg. For example, in every case observed, a vortex configuration can be seen in each blastomere during most of the cleavage process (Plate II, *B, C, D, E*). The direction of the whorl of this vortex is exactly the same as that exhibited by the astral rays of *Astriclypeus* eggs (Dan and Dan, 1947; Fig. 7), only the degree of bending of the component lines is much more acute in the *Spirocodon* egg.

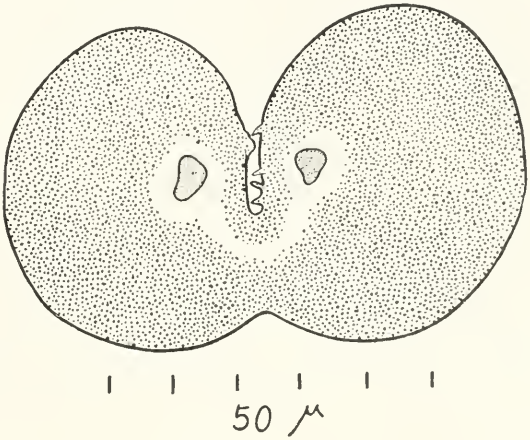


FIGURE 3. *Spirocodon* egg fixed in Flemming's solution showing homogeneous cytoplasm, V-shaped spindle and spinning processes on the furrow sides.

The next fact concerns an utterly new feature of cell activity which finds no similar case among echinoderm eggs. This is the so-called "spinning activity" of the medusan eggs.[2] It has been known for some time that in medusan eggs, after the cleavage furrow advances some distance, fine protoplasmic processes are sent out from the furrow sides to amalgamate into a sheet between the two blastomeres (Yatsu, 1912; Plate II).

As material for observing this spinning activity, *Spirocodon saltatrix* is not particularly favorable, for the processes are so fine that, before amalgamation, they are barely recognizable individually with an oil immersion lens (Plate II, *C′*). But the sheet, once it begins to be formed (Plate II, *D, E*), can be seen with the usual high dry objective (Plate I, *g, h*). Moreover, the partly amalgamated processes can be fixed as such by osmium (Fig. 3). Unfortunately, because of the poor visibility of *Spirocodon* processes, the details of the change from processes to sheet cannot be ascertained. All one can say is that the transformation is rather abrupt and as the sheet is completed, it spreads between the opposed surfaces of the blastomeres like a wetting fluid, sometimes even showing a meniscus at the optical section of its border. As the immediate result, the two blastomeres are pulled closer to each other.

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[2] Andrews (1897) and Moore (1930) discuss spinning activity in connection with echinoderm eggs. Their views will be criticized in a future paper.

The last point concerns the final positions of the divided nuclei. In either unfertilized or fertilized eggs, as long as a nucleus is visible in the living condition, it takes an extremely excentric position toward the animal pole. After it is lost from view and the mitotic spindle develops, sections show that even though the spindle has come back to a more central position, it is still decidedly pushed to the animal pole (compare Plate I*a* and Fig. 2, *3* and *4*). Nevertheless, when the two resting nuclei are reconstituted after cleavage their positions are about midway between the animal and vegetal poles, almost in contact with the furrow sides. This situation is quite different from that existing in *Astriclypeus* eggs, where the nuclei retain the same degree of excentricity until the four-cell stage.

Now let us see how the facts described above can be correlated with the general theory of cleavage developed in the previous papers.

Rotation of the Asters

The direction of the whorl of the vortex configuration in the dividing blastomeres of *Spirocodon* has been shown to be the same as that exhibited by the astral rays of *Astriclypeus* eggs. In the latter case the vortex figure of the astral rays was taken as a direct indication that the asters are turning around their centers (Dan and Dan, 1947). Examination under oil immersion leaves no doubt that the figure in the *Spirocodon* eggs is also primarily due to the bending of the astral rays. Consequently, it is safe to say that the vortices in the two forms are identical in significance. Judging from Yatsu's figure (1912; Plate II, Fig. 19), similar vortices are also evident in the eggs of *Beroë forsikalii.*

Bending of the Spindle

In the previous paper, the primary cause of spindle bending in *Astriclypeus* eggs was attributed to the excentric position of the mitotic figure. It is easy to show that a similar situation obtains in the eggs of *Spirocodon.* In the unfertilized condition, the egg pronucleus is clearly visible in the living cell and it lies immediately beneath the cell membrane at the animal pole (Plate I*a*). After fertilization, although sections show that the first mitotic figure comes back toward the interior to some extent, still it is clearly excentric. As cleavage proceeds, sections also reveal that the spindle is bending. This is true both for eggs fixed with chrome formol (Fig. 2) and for those fixed in osmium (Fig. 3).

Now one is able to understand why the *Spirocodon* eggs flatten during segmentation. In regularly dividing eggs where the spindle simply elongates, the force acts in linear fashion and the cell body can only elongate along the spindle axis. But in the cleavage of medusan eggs, in addition to the force which pushes the two asters apart, there is another force trying to make them turn around their excentric centers. This set of forces determines a plane on which the eggs tend to spread and make flat discs. This fact is most clearly brought out by making contour drawings of the side view of the same egg at various stages and superimposing them. As is shown in Figure 4, the outline of the initial stage can be included within that of the flat stage.[3] When the blastomeres round up again in later stages, the contour becomes smaller once more.

In passing, it must be made clear that the above statement that a set of two forces is at work in the medusan cleavage should not be taken to mean that two different factors are involved in the process. As was discussed in the previous paper (Dan and Dan, 1947), the force causing the rotation of the asters is derived from the elongating force of the spindle as a result of the excentricity of the mitotic figure. Therefore, they are nothing but two fractions of force of a single origin.

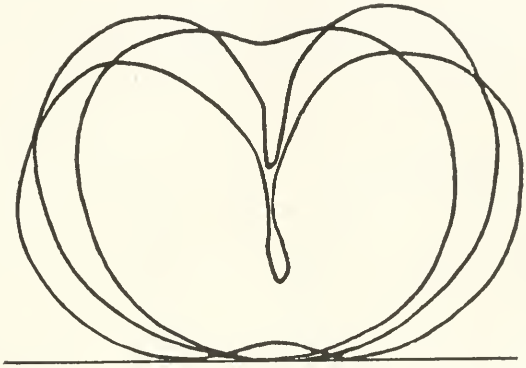

FIGURE 4. Superposition of the contours of the side-view of the same egg in three different stages. The contour of the second stage surpasses that of the first stage both in height and width, indicating that the cell body is spreading flat in this stage. In the third stage, although the width increases, the height decreases, showing that the blastomeres are rounding up.

Returning to the main line of thought, if it be correct that the flattening of the blastomeres and the bending of the spindle are two effects of a single cause, then we should have noticed the same thing in the eggs of *Astriclypeus*. Re-examination of that material shows it to be actually present, as is illustrated in Figure 5. As a matter of fact the bulging out of the vegetal contour was often noticed during that study and its probable cause was attributed partly to spindle bending, partly to astral rotation. The situation would have been grasped more readily if it had been considered in terms of flattening.

SPINNING ACTIVITY AND ITS SIGNIFICANCE WITH RESECT TO CLEAVAGE

Once it is established that astral rotation is one of the important factors in medusan cleavage, one is led to suspect that spinning activity may be closely related as a part of the division mechanism, for when the two asters are simultaneously moving away from each other and rotating around their centers, a tying together of the animal side of the blastomeres would greatly increase the efficacious force available for the tearing open of the vegetal furrow.

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[3] Yatsu's paper includes a somewhat similar figure of the superposition of outlines of successive stages in the cleavage of the eggs of *Beroë*. In connection with this figure, Yatsu is pointing out the absence of lateral elongation in *Beroë* eggs. But it should not be overlooked that the stages he is dealing with are those of the latter half of the division process, and the contour of the early period is not included.

The most direct and decisive way to test this possibility is to interfere with the spinning activity and see whether or not the eggs can divide. A method of mechanical intervention was adopted. It consisted in moving a micro-dissection needle back and forth along the furrow as the latter was being formed. It was thought that this method would prevent the amalgamation of the protoplasmic processes. Special care was taken not to injure the sides of the furrow by the needle during the operation. The result was clear-cut. The eggs divided completely. But on completing cleavage, the blastomeres fell apart. Later it was found that a simpler way to achieve this aim is to make the eggs divide under compression in a hanging drop with an extremely small quantity of sea water. The tracings of photographs shown in Figure 6 illustrate an egg so treated. The reason for the failure of the spinning under compression is probably that, as the egg is flattened so much, the number of the protoplasmic processes which are formed just at the flattened edges of the furrow sides is greatly reduced so that the chance of their meeting and consequently of their amalgamation becomes very slight.

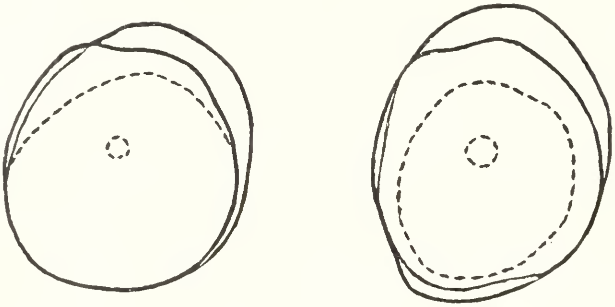

FIGURE 5. Two camera lucida drawings of dividing *Astriclypeus* eggs as seen along the spindle axis. In the left hand one, the furrow is formed only at the animal pole while in the right hand one, the furrow has gone around the cell. The large dotted circles are optical sections of the still uncleaved parts and the smaller dotted circles are cross-sections of the spindle.

At any rate, it can now be said definitely that the sole object of the spinning activity is to tie the blastomeres together and it can be segregated from the process of cell division proper.

PERFORATION EXPERIMENTS

In order to get some insight into the more dynamic phase of medusan cleavage, perforation experiments were made. The method is to take an egg compressed in a hanging drop and punch it through by a micropipette (Dan, 1943*a*).

The results obtained in sea-urchins by this method can be summarized briefly. When a perforation is made through the cytoplasm on the median plane bisecting the karyokinetic figure, the inner border of the perforation is pulled in and becomes a cleavage furrow. If a hole is shifted slightly to the side of the median plane, but still within the crossing range of the median rays (sub-median region), a furrow is formed at the normal position, but simultaneously the perforation is drawn toward the median plane and the two are ultimately united, so that in the end a nearly-normal two-cell stage with a forked furrow results. If the hole is further pushed toward the polar region, it can no longer take part in the furrow formation.

When *Spirocodon* eggs are compressed for punching, because of the excentric position of the mitotic apparatus it is desirable to have the egg axis coincident with the plane of flattening. But unfortunately, even after compressing, the mitotic figure cannot be located definitely because of its poor visibility. Theoretically, the apparent position of the mitotic apparatus under compression can be the most excentric when the egg axis is lying parallel to the plane of flattening and it becomes central when the egg axis is perpendicular to the plane. Another source of ambiguity lies in the fact that when a micropipette punches through the cell in the vicinity of the mitotic apparatus, there is no way of telling to which side of the pipette the mitotic figure will slip away. The final position of the mitotic figure after such treatment in each particular case can be judged to some extent by observing the way in which the furrow appears, but sometimes the authors had to guess it from the later behavior of the perforation.

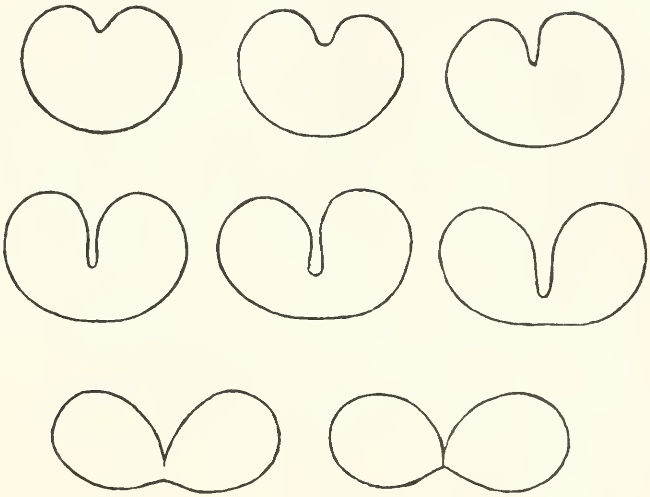

FIGURE 6. Tracings of a series of photographs of a single egg dividing under compression. Spinning has failed and the blastomeres are falling apart. In later stages which are omitted here, they are completely separated. Note, however, that until the cleavage is nearly completed (down to stage 5), the contour change of the blastomere is quite normal, indicating that these shapes are brought about by purely internal causes.

The series of sketches shown in Figure 7 represents the behavior of a perforation on the median plane of the animal side. Just like the corresponding case in sea-urchin eggs, the inner border of the perforation is drawn in, becoming a cleavage furrow, while the median periphery of the cell which would became a furrow under normal conditions remains unchanged. The rest of the division process proceeds in the usual manner. At the fourth stage of the figure, a few partly amalgamated processes are seen and, at the sixth stage, the amalgamation is complete. The division is achieved at the eighth stage. Soon after this, the mitotic figures for the second cleavage are formed and the egg cleaves into three blastomeres. This is instructive in confirming the observation that the first cleavage has failed to divide the cell completely.

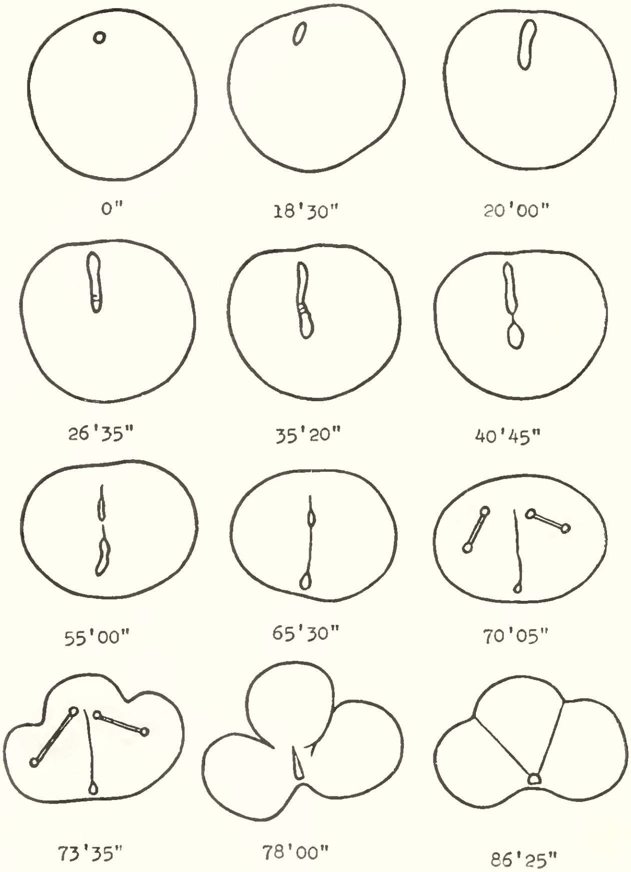

FIGURE 7. Form change of a perforation made on the animal side of the spindle. Note that it makes a cleavage furrow which takes the place of the cell periphery. From the 4th stage on, spinning activity can be seen which succeeds in binding the cells at the 6th stage. At the second cleavage, the larva reaches a 3-cell stage, the middle cell being binucleate and the other two, mononucleate. Temperature, 14.2° C.

The case shown in the next figure (Figure 8) is another example of the perforation of the animal side. In this case, although the result is identical with that of the previous example, as far as the behavior of the perforation is concerned, it is significant in showing a case of the failure of spinning. In this case, since the animal sides of the blastomeres are connected by a protoplasmic bridge, the failure in spinning causes a widening of the tip region of the furrow. As a result, a thread of cytoplasm running between the blastomeres at the vegetal pole is clearly visible. This string of cytoplasm must be homologous to the connecting stalk of sea-urchin eggs and hence it must contain the spindle, or, more correctly speaking, the spindle remnant, within it. In the present case, however, the apparent elongation of the

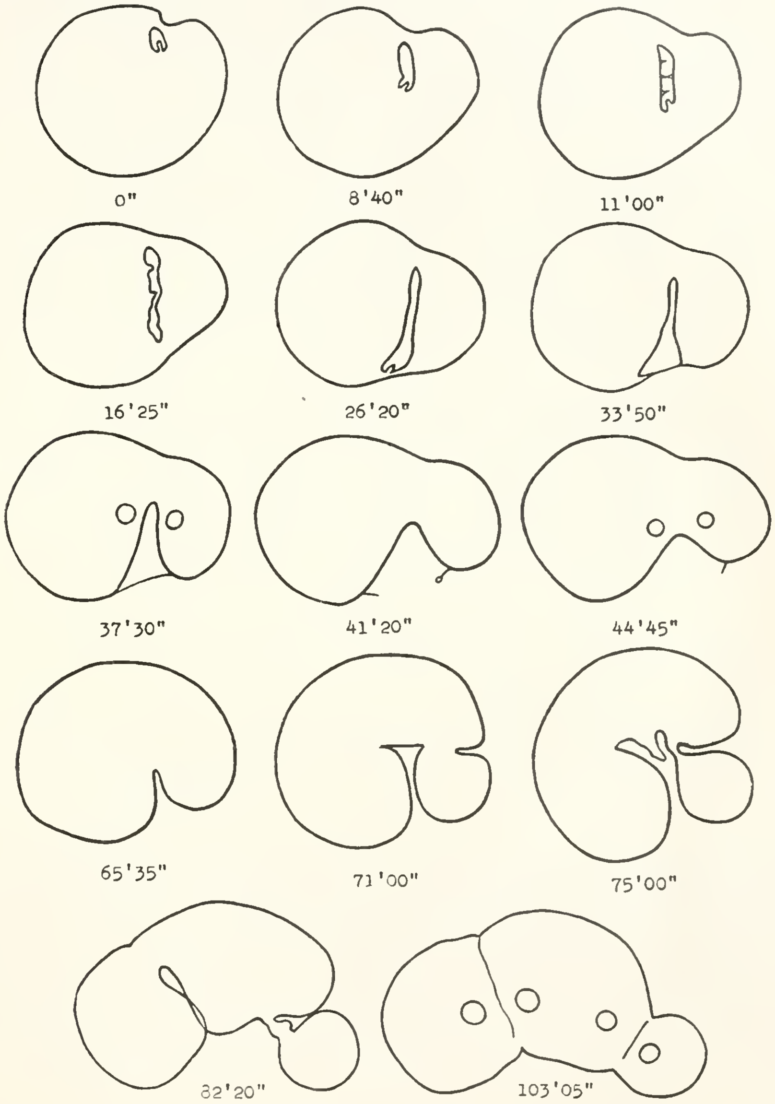

Figure 8. Another example of a perforation in the egg at the animal side of the spindle. At the 3rd stage threads are formed which, however, fail to bind the blastomeres. At the 6th stage, the division process is complete. The protoplasmic string left at the vegetal pole is the "stalk" and contains the spindle. At the 8th stage, the stalk is broken. At the 10th stage, the second cleavage sets in, resulting in a 3-cell stage. Temperature, 10.5° C.

spindle remnant cannot be considered to be an autonomous one but is likely a passive one due to the rounding up of the blastomeres. In the second cleavage, the egg divided into three cells, as in the previous case.

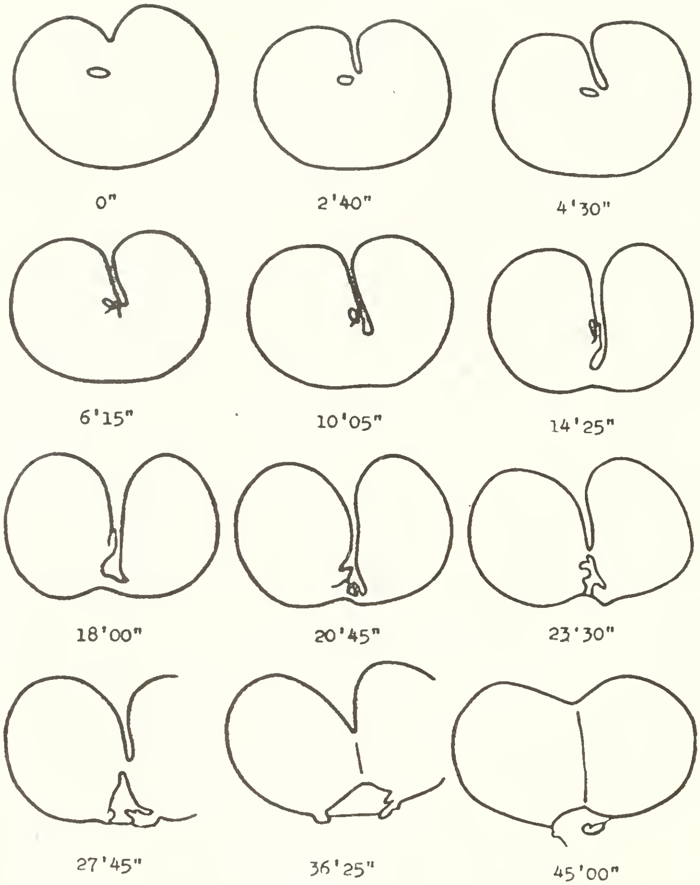

FIGURE 9. The behavior of a sub-median hole on the animal side. Immediately after the operation, the hole was round in shape, but is stretched toward the median plane by the time the 1st drawing was made. At the 4th stage, a protoplasmic protuberance appears in front of the holes. At the 6th stage, the hole is practically fused with the main furrow. At the 7th stage, the hole is stretched, practically becoming a slit so that its fusion with the main furrow cannot be ascertained. But its fusion is evident at the 8th stage through the appearance of a dent on the side of the furrow just above the protuberance. This corresponds to the Y-shaped furrow of *Strongylocentrotus* eggs. At the 9th stage, spinning is achieved at a little higher level than the dent. The spindle remnant is clearly seen at the following 2 stages and is finally broken at the 12th stage as the interkinetic condition is reached. Temperature, 13.3° C.

A typical sub-median case is given in Figure 9. In spite of some irregularities in the contour of the furrow wall, the fusion of the hole with the furrow is evident. The only superficial difference in the sub-median behaviors of the medusan and sea-urchin eggs is probably due to the difference in consistency of the cortex of the two forms.

If a perforation is pushed a little farther from the median plane to the sub-polar region, the hole is only stretched and does not fuse with the main furrow (Fig. 10). If a hole is made in the polar region, it remains quiescent all through the cleavage process (*see* Fig. 14).

In short, as far as the behavior of the perforations on the animal side is concerned, the results are just the same as those in sea-urchin eggs. But once we go to the vegetal side, the reaction changes greatly.

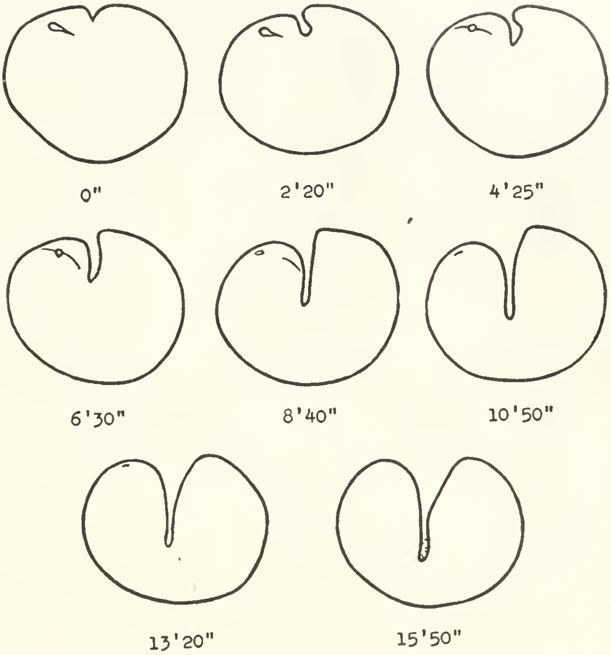

FIGURE 10. A sub-polar hole on the animal side. The hole is pulled toward the median plane. At the 3rd and 4th stages, surface grooves are formed along the direction of strain on both sides of the hole. The hole becomes smaller and slit-like at the 6th and 7th stages and finally disappears at the 8th stage.

The behavior of a median hole on the vegetal side of the spindle is shown in Figure 11. In this case, although the hole is deformed to some extent when the tip of the furrow approaches, there is no sign of the active participation of the vegetal hole in the furrow formation. The situation will more appropriately be described by saying that the furrow advances until it meets a vegetal hole where it simply stops. Therefore, the string of cytoplasm which is found on the animal side in this case must be the spindle remnant.

Here the question arises, whether the behavior of the vegetal hole approaches that of an animal hole if its position is shifted toward the upper pole. Such a case is given in Figure 12. The result is the same as in the preceding case. At the eighth stage of this figure, two nuclei have begun to be reconstructed, but the part of the spindle remnant within the cytoplasm can be recognized as bright streaks which are continuous with the string of cytoplasm outside the cell. The shallow cleavage furrow thus formed is soon abolished completely and a binucleated cell results. At the second cleavage, the cell showed two mitotic figures. In this particular case, the cleavage attempt is unsuccessful in one blastomere and a 3-cell stage is reached at the end.

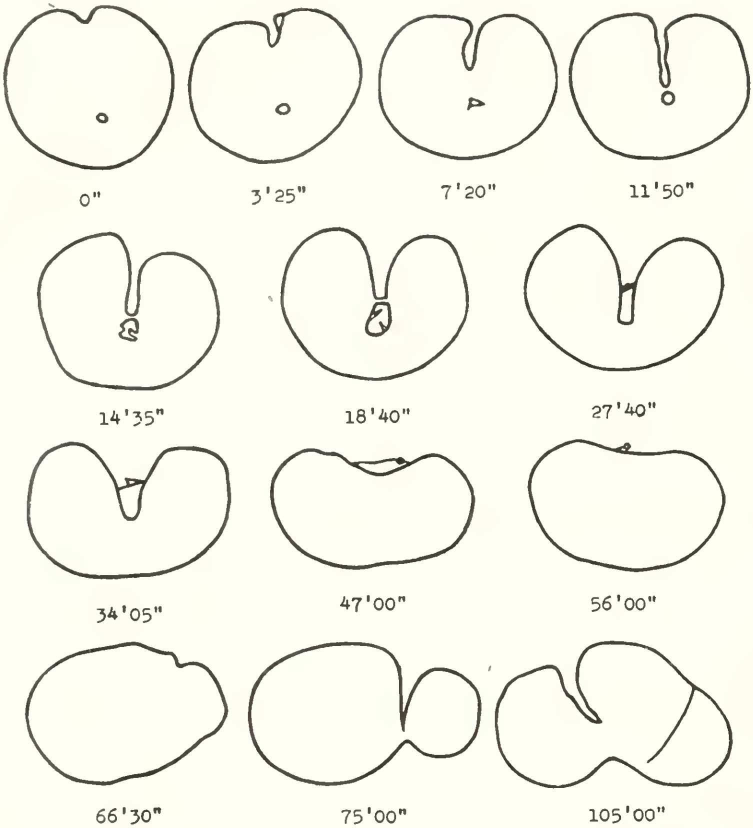

Figure 11. A median perforation on the vegetal side. At the 4th stage, the furrow has advanced so that only the stalk containing the spindle remains between it and the hole. At the 8th stage, the stalk is getting soft. The cell is rounding up at the 9th and 10th stages. The cell divides into three at the 13th stage. Temperature, 12.1° C.

Equally striking is the fact that as soon as a vegetal hole is shifted in position from the median plane, it no longer shows any trace of form change and often vanishes leaving only a scar on the cell surface (Fig. 13). In this egg, the hole is obliterated at the third stage, after which the surface scar left by the hole is represented by a dotted line. The instability of the vegetal hole may indicate the fluid nature of this region because perforations in sea-urchin eggs also become unstable when put in a calcium-free medium.

Combination of two holes on the vegetal side of the spindle does not introduce any new type of behavior. In the case shown in Figure 14, one hole is median while the other hole is polar. The furrow comes down as far as the former and stops there, while the polar hole remains indifferent all through the process. In the case shown in Figure 15, the two holes come pretty close to the furrow, yet they slide along beside it and disappear later.

Summarizing the above results, it can be said that on the animal side of the mitotic figure of the medusan egg, the condition is practically the same as that pre-

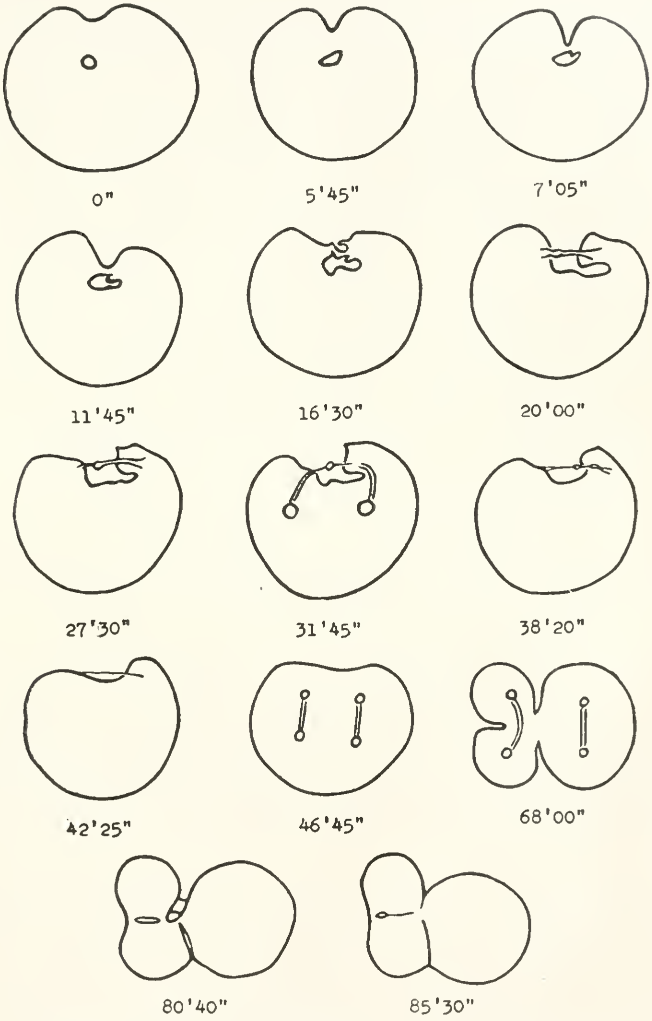

FIGURE 12. A median hole made just below the spindle. The behavior is essentially the same as in the case shown in Figure 11. The 8th stage shows that the string of cytoplasm really contains the spindle. At the 11th stage, two mitotic figures appear. In the right blastomere, the cleavage somehow fails and a 3-cell stage results. The last drawing is significant in showing that the spinning is possible not only between daughter cells but also between adjacent cells which have no common mitotic figure. Temperature, 14.2° C.

vailing in sea-urchin eggs. But the vegetal side is so inert and passive that the situation seems to be utterly different. The impression is unavoidable that the vegetal region is quite fluid. However, if the vegetal region of the medusan eggs is really fluid, is it possible to anticipate a successful cleavage from the proposed theory?

The Division Mechanism of Medusan Eggs

As an introduction to this discussion, let us enumerate important features of medusan eggs which are not met with in sea-urchin eggs, and which, consequently, offer basic materials for the present theoretical consideration. (1) Flattening of the cell and later rounding up of the incipient blastomeres. Astral rotation has been offered as a tentative explanation of this fact. (2) Coming around of the vegetal vacuoles to the animal side in fixed eggs and the shifting in position of the surface marks left by vegetal perforations to the apparent polar region after cleavage (*see* texts of Figs. 13, 15). These two facts seem to have a close bearing on the rotation of the asters. (3) Fluid nature of the vegetal region. The instability of the vegetal perforations and the failure of the vegetal rays to exert stress on the perforations are two basic facts pointing to this conclusion. (4) Movement of the nucleus from the animal side toward the vegetal side during cleavage.

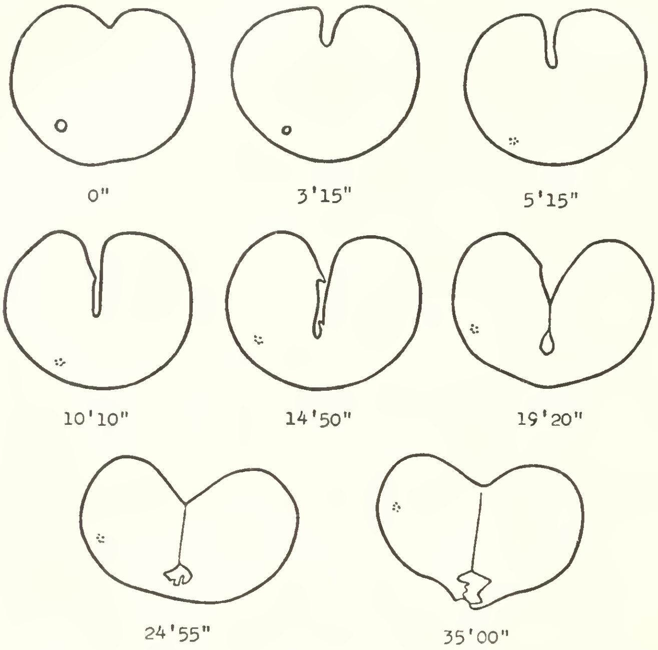

Figure 13. A perforation in the sub-median region of the vegetal side of the spindle. At the 3rd stage, the hole vanishes and a surface scar remains. At the 5th stage, the spinning activity is taking place, and at the 6th stage, the two blastomeres are bound together. At the 8th stage, cleavage is complete. Note that the scar is pushed far to the side, in the polar region. Temperature, 12.1° C.

With the above four points in mind, the authors have arrived at the following conclusion. In the beginning of cleavage, the spindle begins to elongate as in the case of sea-urchin eggs. Since the astral rays of the animal region of medusan eggs are fully effective, this causes first shrinkage and later furrow formation there just as in sea-urchin eggs. As the furrow gets deeper, the median crossing rays are pushed against the spindle (sheath rays) which causes the asters to rotate. This, in turn, makes the cell spread on a plane determined by these forces of elongation and rotation. When the spindle definitely takes a U-shape, the astral centers must be twisted around through nearly 90° from their former directions.

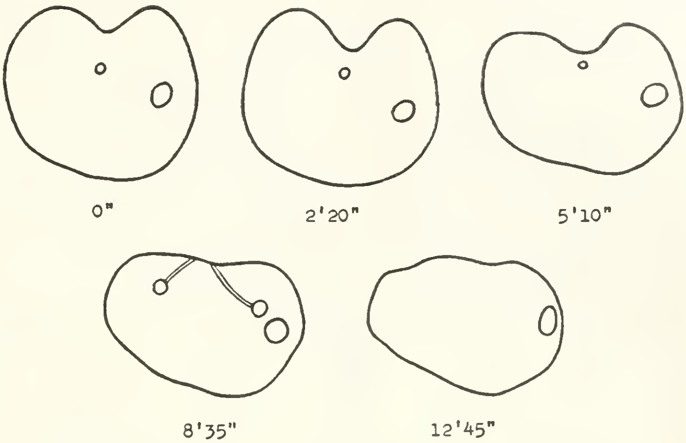

FIGURE 14. Combination of a median and a polar hole. Notice that the polar hole is not affected at all. The behavior of the median vegetal hole remains unchanged by the presence of the polar hole.

Now, if the astral rays of the vegetal region were rigid enough, this condition would immediately cause a fission in the vegetal region between the two asters as in the case of the *Astriclypeus* eggs. But, actually, the vegetal portion of *Spirocodon* eggs is much more fluid and more deformable. Therefore, instead of tearing the vegetal cortex apart (as in typical furrow formation) the cortex and the rays are both stretched out, allowing the central parts of the asters to rotate. In other words, in *Astriclypeus,* the annulment of the strain exerted by the elongating spindle is achieved by the breaking of the cortical layer (furrow formation) while in *Spirocodon* it is accomplished by the yielding of the cortex and the rays to the strain (without furrow formation).

What the authors think about the condition of the rays and the cortex is shown diagrammatically in Figure 16. The cleavage stage corresponds to stage *B* of Plate II. The thin lines represent extremely stretched parts of the rays and of the cortex while the thick ones are the parts which are stretched slightly or not at all. According to our interpretation, there are no rays which go to the furrow side. This ray-free narrow strip lining the furrow side must correspond to the "fan-shaped ray-free area" described in connection with *Astriclypeus* eggs (Dan and Dan, 1947). (In *Astriclypeus* eggs, as the median crossing rays are caught by the furrow head and are pushed in as sheath rays, non-crossing rays on both sides are left behind. As a result, a fan-shaped ray-free gap appears.) In *Spirocodon,* since the arc of the fan-shaped area is stretched out so much, the shape of the fan is greatly flattened. This explains why the daughter nuclei lie so close to the furrow wall. It might be thought that data like those of Figure 16 could be obtained directly by the kaolin method. However, this is impossible since no particles can stay attached to *Spirocodon* eggs.

As the division process approaches its completion and the soft vegetal region is stretched out more and more, the central portions of the asters are released from the strain and regain their former condition. This is expressed as the rounding up of the incipient blastomeres in later stages of the division process. Moreover, such an astral rotation will bring about the rotation of other cell constituents. This must surely be the explanation of the movement of vacuoles and surface scars from vegetal to more animal positions.

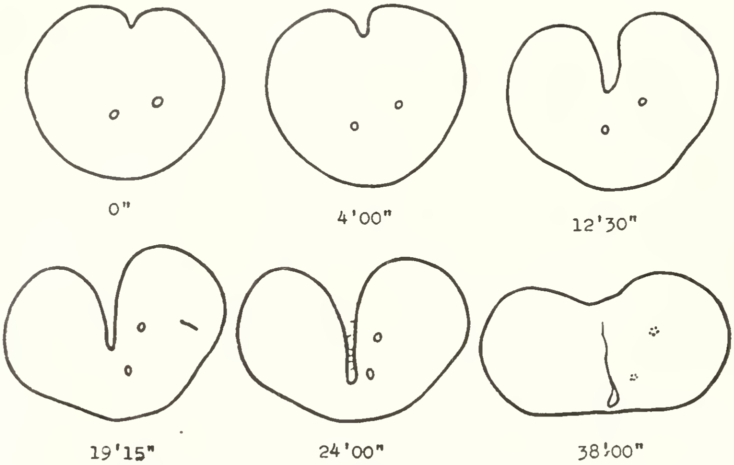

FIGURE 15. Two vegetal holes. The furrow cuts in on one side of the holes. In spite of the fact that the furrow passes quite close to the holes, no change is brought about in their shape. They vanish later. Note also that the direction of the line connecting the two holes is nearly horizontal in the beginning, becoming almost vertical after cleavage is complete. This shows that vegetal cytoplasm before cleavage comes around to the side during cleavage.

However, the last and the most important point is that, as the vegetal material is carried around to the side and even up toward the animal pole, the relative position of the spindle within the cell will become more and more vegetal and finally the base of the U-shaped spindle will reach the vegetal extremity of the cell. In a very rough approximation, it is as if one cuts a medusan egg into two along the cleavage plane, turns the halves by 90° (so that the cut surfaces face downward) and pushes them together animal pole to animal pole. The cleavage is thus complete, with the nuclei in the vegetal region.

When looked at from a different angle, the above theoretical consideration brings forward two more propositions.

(1) It was stated that as the imposed strain is equalized, during the latter half of the cleavage process, the vegetal material is shifted around toward the animal side and the spindle is shifted down. These coupled movements are an action and a reaction. As a result, the authors cannot agree with the opinion that a cleavage furrow strives to reach the opposite pole by its own power.

Such an illusion must have arisen from the fact that when a cleaving medusan egg is observed, the only thing which can be seen moving is the cleavage furrow, and the cell body remains practically stationary. But this is due to a simple physical condition. When a cleaving medusan egg comes to lie on its side as the result of flattening, it must be resting on the substratum by two points, with the center of gravity of the cell somewhere on the line connecting these supporting points. Therefore, no matter what change occurs in the contour, as long as the center of gravity does not change its position, the cell cannot help remaining stationary. The rotational movement of the aster such as was discussed above will certainly not shift the position of the center of gravity very much. In other words, supporters of the opposing theory made an error in selecting the axes of coordinates. They took the more or less stationary cell contour as a reference and compared the movement of the furrow head with it. But if we have to choose a reference point, it seems to us that the position of the nuclei will be a far more reliable one. To illustrate this proposition, a series of camera lucida drawings of a dividing *Spirocodon* egg were superimposed in such a way that the nuclei would coincide as nearly as possible. The result, Figure 17, shows clearly how the vegetal material is carried up toward the animal side. If a dividing medusan egg could be suspended in some medium during cleavage, it might give the same sort of effect.

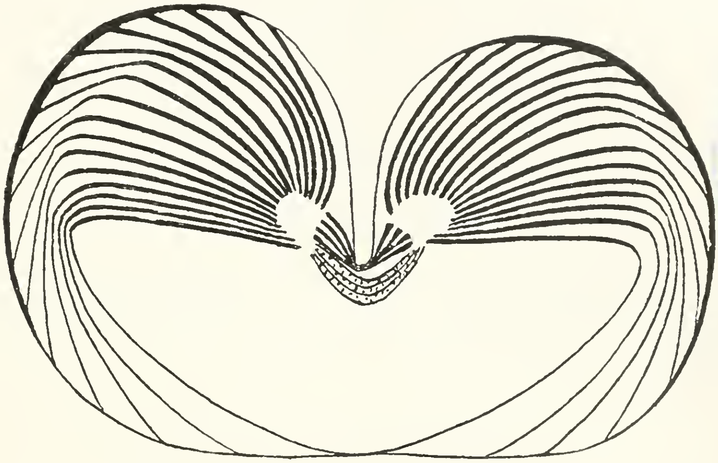

FIGURE 16. The condition of the astral rays and the cortex as interpreted by the authors from stage *B* of Plate II. The distal portions of the vegetal rays are greatly stretched. The animal cortex is drawn into the furrow and the vegetal cortex is stretched to cover the lower half of the blastomeres. Greatly stretched parts are represented by thin lines. The ray-free area is spreading along the furrow wall. This figure is only a rough qualitative representation.

(2) The impetus for the present research was the expectation that the cause for the difference between the modes of cleavage of *Astriclypeus* and *Spirocodon* eggs was probably attributable to the difference in the degree of excentricity of the spindle. But this expectation turned out to be not strictly correct. The accurate statement must be that the difference in the mode of cleavage of the two forms lies in the difference in rigidity of the vegetal halves of the asters. Among *Astriclypeus* eggs, there is, of course, an undeniable correlation between the degree of the spindle excentricity and the degree of the onesidedness of the furrowing (Fig. 1). But this is, in turn, because there also exists a correlation between the degree of spindle excentricity and the degree of deformability of the vegetal halves of the asters.

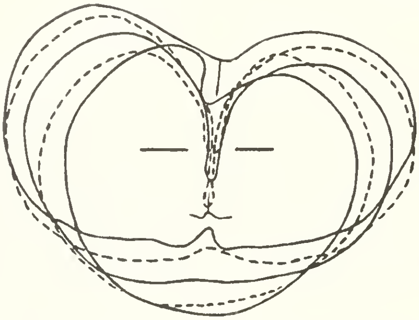

FIGURE 17. The superposition of contour drawings of a dividing *Spirocodon* egg, keeping the nuclei in as nearly a constant position as possible. But the nuclei cannot be made to overlap exactly. Their positions fall on the two lines indicated in the center. The shifting of the vegetal material to outer sides and up to the animal side is evident.

DISCUSSION

Cytoplasmic streaming and astral rotation

Prominent among the theories which have been put forward to explain the division mechanism of medusan eggs is the view that a cytoplasmic current, considered to originate from surface tension changes, is the effective force. This theory seems to have stood partly upon the positive fact that directed movement of the cytoplasmic granules toward the tip of the furrow has been reported ever since the early days of embryology, and partly upon the negative fact that the theory has not been challenged by an alternative one. It certainly created the impression that the phenomenon of cytoplasmic streaming has special prestige in the domain of cleavage studies of medusan eggs and some investigators have even gone so far as to think that it is also true in sea-urchin eggs.

To the authors, however, there seems to exist a profound difference between the streaming in sea-urchin eggs and that in medusan eggs. The streaming in sea-urchin eggs is a current of cytoplasm toward the future cleavage plane, hence it is a current flowing prior to furrow formation (Chambers, 1938; Just, 1939, p. 284). On the contrary, the reports on medusan eggs are all concerned with cytoplasmic currents along the already formed cleavage furrow converging toward the furrow head. Therefore, even allowing for the possibility that streaming is a chief cause of cell division, the modes of action of the two streamings must be entirely different.

Moreover, the literature on medusan cleavage is further complicated by the existence of ectoplasmic movement. As is known, the ectoplasm of *Beroë* eggs changes its thickness and usually thickens around the cleavage head (Spek, 1926). Since the ectoplasm accumulation occurs at the point toward which the cytoplasmic currents are directed, the former is often thought of as the result of the latter. But these two ought to be clearly differentiated. In short, within the vague term of "cytoplasmic streaming," three different things are included: namely, sea-urchin egg streaming, medusan egg streaming and the medusan ectoplasmic movement.

As for the streaming of sea-urchin eggs, the senior author has offered the interpretation that it is a passive movement of the fluid cytoplasm to fill in the gap formed between the separating asters. Concerning the streaming of medusan eggs, the authors are still more skeptical. They think that this streaming itself might be an illusion due to the *en masse* rotation of the asters. It must be remembered that when other investigators talk about a cytoplasmic current, what they are actually seeing is a movement of suspended granules. It is proposed that the mere fact that the granules move in reference to some landmarks outside the egg cannot have finality in the conclusion that the cytoplasm is flowing. The conclusion is allowable only when the granules move in reference to other cell structures such as the cell membrane. In the case of sea-urchin eggs, such a guarantee is provided because the streaming, after having reached the site of the future cleavage plane, is said to change its course and go inward. Since no such guarantee is available in the case of medusan eggs, who can say that it is only the cytoplasm which is moving? Furthermore, if it is a real streaming, why is there not found an accumulation of granules beneath the furrow head where the ectoplasm is thick?

According to Spek (1926), the cytoplasmic currents of *Beroë ovata* flow along the furrow sides, meet at the furrow tip and go a short distance to the inside along the median plane. This fact, he contends, is an evidence that the higher surface tension at the furrow head is causing the one-sided furrow formation. But to our surprise, on reading his paper carefully, we do not come across any streaming in the strict sense. The arrows indicating the direction of the flow in his text figures are only representing the directions of an hypothetical streaming *if such a streaming could be considered to be the cause of the movement of the green ectoplasm.* On page 60, he writes, "Während dieser Substanz Verlagerungen konnten Plasmaströmungen direkt nicht wahrgenommen werden. Konstruirt man aus dem Effekt der Plasmaverlagerungen das Strömungsbild, so erhalten wir ein eindeutiger Weise einen oberflächlichen Zustrom nach der Furche und einen axialen Abstrom von derselben Weg (S. Textabb. 3)." In other words, this is only a "Strömungsbild" where no streaming is seen. But from the authors' standpoint, the fact that his arrows are indicating the exact directions of the rotation of the asters is too significant to be overlooked.

Ectoplasmic movement and aster rotation

As for the movement of the ectoplasmic layer of *Beroë ovata,* the authors are of the opinion that this is again a passive movement. The ectoplasmic thickening at the beginning of furrow formation must be the result of the shrinkage of the furrow surface at this stage as has been observed so many times in the kaolin experiments of sea-urchin eggs. The accumulation of the green ectoplasmic material at the points of the last connection between the blastomeres can be accounted for as follows. When the vegetal pole is stretched out and is about to be torn, the connecting bridge between the blastomeres must almost exclusively consist of the cortical material without including fluid endoplasm. Therefore, when it finally gives way, there ought to be an accumulation of the green material. During the intermediate stages, the surface area of the furrow region shrinks slightly (from unpublished data on sea-urchin eggs). Consequently, the ectoplasm around the furrow head can retain the thickness illustrated in Spek's figures.

But far more important is the later behavior of the green ectoplasm. According to Spek, in the first two cleavages, the green material which accumulates around the point of the last connection once more distributes itself evenly around the blastomeres. But at the end of the third cleavage, its re-distribution is prevented and even the nuclei are held there. As a result, at the fourth cleavage, the furrow starts to be formed at the opposite pole of the larva (the micromere pole). But this cleavage and the following one (the fifth) being extremely unequal, two sets of micromeres consisting entirely of the green material are formed. These micromeres are the mother cells of the future combs. The macromeres above them are still retaining the residue of the green material adjacent to the micromeres. In other words, as long as the cleavage is extremely unequal, the green substance stays on the same side of the larva.

After a long resting period extending for nearly two hours, the macromeres go into a division. This division is equal and the furrow starts to cut in from the micromere pole. As the furrow advances toward the other side(the polar-body pole), the last trace of the green material is carried away with it and at the completion of the cleavage, the green substance is found at the other (polar body) pole. In the following extremely unequal cleavage all the green material is given off as micromeres. As is clear, this new set of micromeres lies opposite from the group of comb-forming micromeres and they are endodermal elements.

From the authors' concept of medusan cleavage, this back and forth movement of the green ectoplasm is not surprising, since medusan blastomeres are turning somersaults at each division. When the division is unequal, although the same turning around accompanies it, the division cannot take the ectoplasmic material across the large cell and apparently leaves it in place. So it must always be an equal division which can transfer the material across to the opposite side.

In the field of amphibian embryology, the opinion is often expressed that the process of amphibian gastrulation is a formative movement by which various mesodermal elements are delivered to their definitive positions. In that sense, it is exceedingly interesting that the cleavage process itself is acting as a formative movement in *Beroë*.

The reason why the green ectoplasm re-distributes itself evenly after the first two cleavages is unknown to the authors. Spek thinks that it is due to the low plasma-viscosity during these mitoses. Whatever the explanation is, the phenomenon of the complete re-distribution of the ectoplasm seems to have a close bearing on the fact that the cleavage furrows cut in from the animal pole (the polar-body pole) during this period. In *Spirocodon saltatrix*, on the other hand, the redistribution is prevented even for the first cleavage. As a result, the nuclei come down half way between the two poles, lying quite close to the furrow surface, and the cleavage furrow of the second division starts from there. This is functional evidence that the animal pole has come around to the furrow side and the vegetal material is spread out to cover the lower half of the blastomeres.

Medusan embryology and aster rotation

The concept of the rotation of the blastomeres of medusan eggs seems to offer a hint with respect to a more general problem of the embryology of ctenophores. Among this group, it has been a puzzling fact that the egg axis and the larval axis run in opposite directions. This means that the apparent vegetal pole of the egg where the comb-forming micromeres appear becomes the fore-end of the swimming larva. This is quite contrary to the situation found in other animal phyla. For this reason, investigators who put emphasis on the later development name the two poles of ctenophore eggs exactly opposite to the way it is customarily done, calling the polar-body pole the vegetal pole (Schleip, 1929; Korschelt, 1936). Korschelt even goes so far as to illustrate the eggs up-side-down. All these confusions have their origin in the fact that people view the development of ctenophores with the conventional idea that the egg axis should coincide with the larval axis. But if the blastomeres turn upside down at each division, the egg axis changes its direction each time. In other words, among medusae, the egg axis seems to have little meaning.

SUMMARY

1. Characteristics of division involving an excentric mitotic figure: namely, astral rotation and spindle bending, are recognizable in the division of *Spirocodon* eggs.

2. Perforation experiments indicate that although the physical condition of the animal region of *Spirocodon* eggs is similar to that of sea-urchin eggs (*Pseudocentrotus depressus*), the vegetal region is quite fluid and deformable.

3. In *Astriclypeus*, the vegetal region is like the animal in physical consistency so that the vegetal region is pulled open (i.e., furrow formation takes place) sooner or later. In *Spirocodon*, the vegetal region is quite fluid. As a result, it is never pulled open but is simply drawn out until it finally breaks. This is the fundamental difference between heart-shaped cleavage and medusan cleavage.

4. Astral rotation is discussed in connection with cytoplasmic streaming, ectoplasmic movement and medusan embryology generally.

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PROGRAM AND ABSTRACTS OF SCIENTIFIC PAPERS PRESENTED AT THE MARINE BIOLOGICAL LABORATORY, SUMMER OF 1947

General Meetings

A delayed effect of X-rays on pepsin. Rupert S. Anderson.

It is well known that X-rays inactivate enzymes such as pepsin. Determinations of the activity remaining are usually carried out as soon as the period of irradiation is completed.

With pepsin it has been noticed that in addition to the immediate effect there is a slowly developing further inactivation. This inactivation occurs when the irradiated enzyme stands in a buffered solution at pH 5.3–5.4. Ultimately the total inactivation was several times as large as that observed initially.

It has not been possible to imitate the delayed inactivation by incubating pepsin with concentrations of hydrogen peroxide up to one millimolar. Moreover irradiated solutions which have been dialyzed against the original pure buffer solution, to restore the initial conditions as closely as possible, still show the delayed inactivation. The generality and possible significance in biological radiation experiments of this type of phenomenon will be further studied.

On the occurrence of myoglobin in Busycon caniculatum. Eric G. Ball and Octavia Cooper.

In contrast to the bulk of muscle in *Busycon caniculatum*, the protractor and retractor muscles of the odontophore and the radula are bright red in color. This suggests the presence in these muscles of an iron porphyrin compound. The positive identification of this muscle pigment as an iron compound seemed of particular interest in view of the fact that this animal possesses a copper blood pigment, haemocyanin, which displays an affinity for oxygen greater even than that of mammalian myoglobin.

A total of 86 animals yielded 74.2 grams of wet muscle. These were ground in a mortar with sand and water, the mixture centrifuged, and the turbid red supernatant brought to 60 per cent saturation with $(NH_4)_2SO_4$. After standing overnight the colorless precipitate was centrifuged off and the pigment precipitated from the supernatant by the addition of $(NH_4)_2SO_4$ to 90 per cent saturation. The bright red precipitate was removed by centrifuging or filtration.

The precipitate dissolves readily in water or neutral phosphate buffer to yield a clear red solution. Its absorption spectrum shows sharp peaks at λ 576, 540, 415, and 270 mμ, and thus resembles hemoglobin. The ratio of the extinction coefficients for 540 : 576 is 1.15 and for 415 : 540 it is 9.3. In hemoglobin the comparable ratios are 0.96 and 8.7.

The pigment reacts with $Na_2S_2O_4$, ferricyanide, and CO to form compounds analogous to those produced when these reagents react with hemoglobin. The CO compound shows absorption peaks at λ 569 and 538 mμ. The span of 70 Å in the α band between the O_2 and CO compounds indicates a strong affinity of the compound for CO. The iron content of a salt free dry preparation is 0.17 per cent.

A crystalline chlorohemin derivative can be prepared. The absorption spectra of the ferrihemehydroxide, ferrihemecyanide, and ferrohemecyanide compounds are identical with those yielded by blood hemin.

Physiological studies on the mechanism of action of DDT in insects. John B. Buck and Margaret L. Keister.

Weight changes and oxygen uptakes were measured in adult blue bottle flies, *Phormia regina*, in high, and in low humidities, after the flies had walked on a microcrystalline surface of DDT for 15 minutes. Initial percentage body weight fractions were: water, 65; respirable substrate, 20; irrespirable material, 12.5; skeleton, 2.5. Weight loss was nearly linear in all four groups of flies during the first 10 hours. Water loss, in per cent of original weight, totaled: dry DDT, 25.6; dry control, 14.6; wet DDT, 14; wet control, 6.5. Substrate (dry weight) loss, in per cent of original weight, over the same 10-hour period, totaled: dry DDT, 4.4; dry control, 2.4; wet DDT, 10; wet control, 1.5. Per cent substrate loss to death (18 hours for dry DDT; 8 days for wet control) totaled: dry DDT, 6; dry control, 15.2; wet DDT, 15.5; wet control, 20. Average oxygen uptake in mm.^{3}/hr./mg. fresh weight was 2 in both control groups, 10 in the wet DDT flies between 4 and 8 hours after poisoning, and a maximum of 7 at 4 hours in the dry DDT flies. Individual poisoned flies briefly reached peaks up to 15 times the control rate. Similar values were also reached by rare controls which were active instead of motionless. R. Q. was about 1 in the poisoned flies, 0.8 in quiescent controls. Total oxygen uptake per individual poisoned fly varied directly with both fresh and dry weight losses. Poisoned flies could accumulate oxygen debts.

Conclusions: (1) DDT enhances both water and substrate loss. (2) Low humidity reduces the respiratory stimulation caused by DDT. (3) DDT has no specific effect on either anaerobic or aerobic glycolysis, the observed respiratory and weight changes being indirect, and attributable rather to hypermotor activity. (4) Death from DDT is not primarily due to either water loss or exhaustion of respirable reserves.

Direct determinations of internal hydrostatic pressure of Fundulus egg. W. BUNO AND ROBERT CHAMBERS.

By the use of mercury manometer it has been possible to measure directly the hydrostatic pressure within the Fundulus egg which has a diameter approximately 2 mm. It was found that there is an immediate response of the egg to changes in the concentration of a balanced salt solution, namely, S. W. There is a rapid exchange of water and of salts, the exchange of salt being slightly slower. Our chief contribution is that such exchange occurs in a solution containing all the electrolytes of sea water. In preliminary experiments it was found that the addition of 5 per cent alcohol to sea water caused the pressure to fall immediately, followed by a rise to its original value and sometimes exceeding the value. This was repeated several times with similar results on the same egg in the same environment in which the concentration of the alcohol approached 30 per cent.

Toleration of lowered oxygen tension by cave and stream crayfish. W. D. BURBANCK, JOHN P. EDWARDS AND MADELINE P. BURBANCK.

Limestone caves are abundant in southwest Missouri and from many of them issue streams which eventually flow into the White River or its tributaries. Smallin Cave in Christian County, Missouri, sixteen miles from Springfield, is such a cave and is peculiar in two ways. First, the mouth of the cave is one of the largest in the United States measuring about ninety-eight feet wide by sixty feet high. Secondly, in the space of three-quarters of a mile, the cave tapers to less than ten feet and opens into a deep sinkhole, a condition allowing much organic material in the form of leaves and soil to enter the cave.

In Smallin Cave is found the white blind crayfish, *Cambarus setosus*. Directly outside the cave and living under practically the same conditions of temperature, dissolved oxygen, pH, and total hardness of water, lives the stream crayfish, *Cambarus rusticus*. Since the surface drainage is a factor in the total flow of water in Smallin Cave, the cave crayfish at times may live in a torrent, a swift stream, or in isolated pools. During periods of drought, the stream issuing from the cave may dry up and the stream crayfish disappear.

Following the technique of Park, Gregg, and Lutherman (*Ecology*, 21; 1940), in a series of three experiments, thirty-three stream and thirty-three cave crayfish were placed three each in liter flasks of boiled cooled water ($O_2 = 4.0715$ cc. per liter) sealed with mineral oil. Following the death of all of the animals, oxygen analyses by the Winkler method showed that the cave and stream crayfish reduced the water to approximately the same oxygen tension—no significant difference between 0.1978 and 0.2452 cc. per liter respectively. The cave crayfish lived longer than did the stream crayfish, however. Average survival time for cave crayfish was 829.9 ± 35 minutes and for the stream crayfish 272.3 ± 21.5 minutes. Thus, the longer-lived cave crayfish seem to have a lower rate of metabolism than the stream crayfish.

The shape of oil drops injected into the axoplasm of the giant nerve of the squid. ROBERT CHAMBERS.

The sheath of the fiber is relatively easy to puncture with a slanting thrust of the micropipette, the aperture of which is in the neighborhood of 1 to 2 microns in diameter. By this means it was found possible to introduce oil drops into various depths of the interior of the fiber. The oil drop assumes immediately an ovoid shape along the long axis of the nerve. Agitation of the needle inserted in the vicinity of the injection changes the consistency of the axoplasm so that the oil assumes a spherical shape. The linear arrangement of the material composing the homogenous appearing axoplasm is also indicated by subcooling the fiber to − 3° C. and inducing freezing of the axoplasm by inserting a needle. The icicles which form are cylindrical rods parallel to one another and to the long axis of the fiber. Colorimetric determinations indicate a pH of 6.8 for the normal axoplasm. When the axoplasm is liquified by damage the pH changes quickly to the neighborhood of 5.4. These experiments indicate that the axoplasm consists of ultra-microscopically arranged material, presumably in a gel state, which reverts on injury to the sol state. The pH changes observed are those of living protoplasm. This is in direct contrast with effects observed in the yolk of the Fundulus egg which exhibits no pH change when mechanically disturbed.

Deformation delay in Arbacia ova centrifuged in caffeine solutions. RALPH HOLT CHENEY.

Typically, unfertilized Arbacia ova, when centrifuged at 10,000 × gravity for 7 minutes, become elongated and break into halves and even quarters with more prolonged centrifugation. Sea urchin eggs in caffeine solutions resist changes in their spherical form under centrifugal force of that magnitude. For comparison, control eggs in sea water were centrifuged with eggs from the same female in 0.02 per cent, 0.10 per cent, and 2.0 per cent caffeine-in-sea-water. The response of ova when subjected to the 0.10 per cent CSW (M/200) was noted particularly because that is the concentration which has been shown by the author to definitely influence cellular metabolism.

All concentrations prevented deformation of the caffeinized eggs when centrifuged at 10,000 × g. for 7 minutes. Stratification of the echinochrome "appeared" more compact in caffeinized ova. Twelve minutes of centrifugation resulted in the breaking of 100 per cent of the controls whereas deformation and breaking in the experimental series of 0.02 per cent CSW to 2.0 per cent CSW varied from 12 per cent to 0.08 per cent of the eggs. Increasing the caffeine concentration decreased the percentage of breaking and degree of deformation.

Centrifugation at 3,000 × g. for 3 minutes resulted in no deformation of either the controls or experimentals and stratification of pigment was equal. This latter observation indicated that the "apparent" difference in stratification under greater centrifugal force, resulting in the elongation of the control eggs, was not due to any change in viscosity. It could be accounted for by the difference in the distance of sedimentation in the deformed controls and the spherical experimentals. Evidence that resistance to deformation is due to the effect of caffeine on the tension forces at the surface layers is now under investigation.

Experiments on germinal localization in Ilyanassa. A. C. CLEMENT.[1]

If eggs of Ilyanassa are removed from their capsules and grown in ordinary filtered sea water, they succumb to infection within a few days without attaining the veliger stage. With a semi-sterile technic, including the use of Pasteurized sea water, the naked eggs develop normally. Using this technic, the developmental capacity of various fractions of the egg has been tested. The following observations are based mainly on a study of living material.

The polar lobe is necessary for normal development. If the lobe is removed at the trefoil stage of the first cleavage, a partial larva results. Elements of the velum, including the large cilia, appear regularly and eyes are often formed. Otherwise, the larva bears little resemblance to the normal veliger, either in general form or in recognizable larval organs.

The polar lobe normally passes into the D quadrant at the second cleavage. If the D blastomere is punctured and removed, the ABC fraction produces a deficient larva resembling the lobeless one. In contrast with this, removal of either the A, B, or C quadrant from a whole egg often results in a larva which, although showing certain deficiencies (particularly of the velum), is in most respects similar to the normal one.

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[1] Aided by a grant from the Carnegie research fund of the College of Charleston.

If the D macromere is punctured and removed after it has given rise to three ectomeres and the mesentomere cell 4d, development is virtually normal in most cases. The only regular deficiency detected is a reduction in size of the larva. Much of the material originally contained within the polar lobe, and later the D quadrant, is removed in this operation. It thus appears that the original lobe region loses its essential morphogenetic value during the cleavage process, presumably through an allocation of potency to some or all of the first four cell derivatives of the D quadrant.

The cytological basis of salt excretion from the gills of Fundulus heteroclitus. D. EUGENE COPELAND.

Fundulus heteroclitus were subjected to abrupt changes from sea water to tap water and vice versa to see if the cellular mechanism for salt excretion could be demonstrated in the gills. A columnar type acidophilic cell extending from the basement membrane to the surface of the gill filament shows a secretory vesicle at the distal end in sea water and none in fresh water. The cell is rich in very fine, filamentous (possibly oriented granules) mitochondria in both sea water and fresh water. Silver nitrate tests for chloride show a very marked response in the secretory vesicle and to a less extent in the body of the cell. Fresh water adapted cells show little or no chloride. In several distilled water adapted cases, the cells rounded up and left both the free surface and the basement membrane.

Inhibition of Arbacia egg cleavage by podophyllotoxin and related substances. IVOR CORNMAN.

The effects of podophyllin upon *Asterias* eggs are reported in another abstract in this issue. Podophyllin is a mixture of podophyllotoxin (m.w. 414), quercetin (m.w. 321), podophyllic acid, and unknown constituents. The effect of podophyllin on *Arbacia* egg cleavage appears to represent the combined effects of podophyllotoxin and quercetin, revealing a wide range of concentrations (0.4–2 mg./L) over which it slows cleavage slightly, then a sharp threshold at which cleavage is completely inhibited (4–6 mg./L). Like podophyllotoxin, podophyllin completely inhibits the cleavage of some eggs at concentrations which only moderately delay the cleavage of the remainder. Quercetin (20 mg./L) more than doubles the time required for cleavage while permitting all cells to cleave, and delays cleavage time 10 per cent at a much lower concentration (0.6 mg./L). Podophyllotoxin delays cleavage time 10 per cent at 0.07 mg./L and blocks completely at 0.5 mg./L. Thus these two compounds are more active than colchicine (m.w. 399), which acts at 10–80 mg./L. Rutin, which contains a quercetin nucleus (m.w. 664), delays cleavage very slightly at saturation.

Other visible processes, e.g., activity of sperm and lifting of the fertilization membrane, proceed normally in podophyllin at concentrations as high as 100 mg./L. The cleavage furrow is unaffected, as evidenced by cleavage of eggs which have reached anaphase at the time of exposure to 0.5 mg./L of podophyllotoxin.

Two other echinoderms are more sensitive to podophyllotoxin than is *Arbacia*. The maturation of *Asterias* eggs is blocked completely at 0.01 mg./L. Cleavage of *Echinarachnius* eggs is slowed at 0.01 mg./L and blocked completely at 0.04 mg./L.

The distribution of phosphorus in the unfertilized egg of Arbacia punctulata. ROBERT K. CRANE.

Mature, unfertilized eggs from freshly collected Arbacia contain a total of 130 milligrams phosphorus (Fiske and Subbarow) per gram nitrogen (Folin), or 0.31 milligram per cent of wet weight.

Preliminary partition (expressed in milligrams phosphorus per gram nitrogen): *acid-insoluble*, 35 (range 31–39); *acid-soluble*, 53 (range 47–60); *ether-soluble*, 53 (range 40–66). Separate samples were used for each fraction.

Fractionation of acid-insoluble (Schmidt and Tannhauser, *Jour. Biol. Chem.*, **161**; 83, 1945): phosphoprotein, 27; ribonucleic acid, 6.5; and desoxyribonucleic acid, 1.5.

Analysis of total acid-soluble was made on trichloracetic acid filtrates. Since echinochrome interfered with analysis of orthophosphate, an ammonium sulfate extract (Lowry and Lopez, *Jour. Biol. Chem.*, **162**; 421, 1946), free of echinochrome and proteins, was prepared. This yielded 35 milligrams phosphorus per gram egg nitrogen from suspensions which had total acid-soluble of 50; of the 35, 11 were inorganic (Lowry and Lopez), 14 were liberated by 7-minute hydrolysis in 1 N hydrochloric acid at 100 degrees centigrade, 10 more by 100-minute hydrolysis. Total content cannot be analysed in ammonium sulfate solutions.

For unfertilized eggs, a nitrogen ratio of 100 milligrams per gram dry weight has been reported. Suspensions in July (fresh urchins) confirmed, but those prepared in August (stored urchins) showed ratio as low as 55.

Total phosphorus ratio (dry weight) increased with lower nitrogen. At nitrogen ratio of 55, total phosphorus approximated 17 instead of 13 milligrams per gram dry weight. Significant changes also occurred in fractions.

Logarithmic curves relating total and fractions of phosphorus to nitrogen (dry weight basis) yielded straight lines. Extrapolation to 100 milligrams nitrogen per gram dry weight showed 13 milligrams total phosphorus per gram dry weight (0.1 × value based on nitrogen). Data were insufficient for similar derivation of fractions. In the future, data will be thus graphically analyzed in belief that truer values than afforded by averages will be obtained.

The author worked in the laboratory of Dr. Clowes and is grateful for many helpful suggestions.

A preliminary study of the vertical distribution of marine organisms in the Cape Cod area. ELIZABETH M. FAHEY.

Intertidal marine organisms appear at low tide to be arranged in a number of superposed zones. Transects across these zones from the highest levels in which marine organisms appear to the lowest levels to which the tides recede have been studied, with the vertical distribution of the organisms found thereon recorded. Though the overall heights of the transects vary with the mean tide range from place to place, the data gathered from Plymouth, Provincetown, Lewis Bay (Hyannis), Nobska Point, Penikese Island and in Woods Hole correspond well as to the number of zones and in their approximate proportion to the height of the transect. Likewise, from place to place, these zones have had, at the same time of the season, about the same floristic and faunistic constitutions and the same positions in relation to each other.

Data from a study of the repopulation of denuded transects at Nobska Point support the hypothesis that the organisms "seed" onto the rocks throughout a greater vertical range than the same species occupy as adults. The primary repopulating macroscopic forms were *Littorina littorea* and species of *Calothrix, Enteromorpha,* and *Polysiphonia.*

Interaction between the implanted embryos and the internal organs of an adult teleost, Opsanus tau. PAUL S. GALTSOFF AND EUGENIA GALTSOFF.

Embryos of *Opsanus tau,* from 3 to 5 mm. long, introduced with aseptic precautions intraperitoneally into an adult toad fish survive and attach themselves to the abdominal wall, liver, intestines and possibly to other organs. Normal development of the implanted embryo ceases, but the embryonic tissues continue to grow in an erratic manner forming teratomas. Embryos attached to the surface of the liver produced metastases which invaded and destroyed the underlying portions of this organ. Three week old tumors, growing in the liver, consisted of easily recognizable elements of connective tissue, blood, notochord, cartilage, and muscle fibers. No traces were found, however, of the epidermis and typical mucus cells present in the normal embryos.

One encapsulated teratoma, attached to the peritoneum of the abdominal wall, produced no metastases. It consisted of various embryonic tissues and organs irregularly scattered within the capsule.

None of the eight fishes used in the experiments died as a result of the operation. The behavior of the operated fishes appeared to be normal.

From a preliminary study of the experimental material conclusion is drawn that the regulatory mechanism responsible for the orderly development of the embryo ceases to function when the latter is implanted in the organs of an adult organism. Erratic growth of some of the embryonic tissues continues, however, and results in the destruction of the cells and tissues of the host.

Altering the expression of position effect. W. Goodsmith and Taylor Hinton.

Plum eye color in *Drosophila melanogaster* is due to a complex inversion in the second chromosome. This "position effect" when crossed to most stocks does not vary in expression. However, when crossed to Inversion (2R)NS, offspring appeared with reddish, almost wild-type eyes and exceptional mottling of the pigment. An attempt was made to ascertain whether it was the inversion causing the modification of Plum or whether it was some other factor in that stock. Therefore, Inversion (2R)NS was extracted from the original modifying stock and incorporated into a new stock containing Inversion (2L)t, which does not modify Plum. This stock, In(2L)t, In(2R)NS/Ins(2L + 2R)Cy, was crossed to Plum and the majority (906) of the offspring could be placed into 2 classes both phenotypically and cytologically. These were: Ins(2L + 2R)Cy/Plum, giving a normal Plum eye color, and In(2L)t, In(2R)NS/Plum, giving a modified expression of Plum as previously described. Two additional types were produced (34 individuals): one, a normal Plum without the Curly phenotype, and the second, a modified Plum with the Curly phenotype. Cytological analysis has shown these to be In(2L)t, In(2R)Cy/Plum, and In(2L)Cy, In(2R)NS/Plum, respectively. Therefore it can be concluded that the modifying effect is correlated with the presence of the (2R)NS inversion. It is conceivable that it is not the inversion *per se* but rather genetic modifiers within the inversion which are responsible for the effect. However, a series of chromosomal rearrangements recently obtained by irradiation include 3 which have the same effect upon Plum as does In(2R)NS. It would be too coincidental that in all of these a genetic modifier mutated simultaneously with the occurrence of a chromosomal rearrangement. Therefore, it is suggested that in several cases a chromosomal rearrangement has modifying effect upon another chromosomal rearrangement. Tentatively, this might be termed a "position effect upon a position effect."

Conditions affecting development of the male phase in Crepidula plana. Harley N. Gould.

In previous studies the author concluded that development of the adult male phase in the sexual cycle of this species necessitated close association, but not contact, of the small potential males with larger individuals of the same species. W. H. Coe has since stated, however, that "neither in *Crepidula plana* nor in any of the other five species of that genus studied is association with other individuals a prerequisite for the development of the functional male phase." To test this matter further, very young spat of *Crepidula plana* of one to five millimeters shell length which had not yet developed any external indication of sex were removed from the clusters growing in hermit crab shells and allowed to live in glass dishes with running sea water, free from any influence of larger individuals.

Over 400 such specimens have been examined at ten-day intervals. Over 100 have to date been followed for more than forty days. Many have attained and passed the average size of males. No adult males have appeared in any of the cultures. An incomplete development of maleness, indicated externally by a stumpy rudiment of the penis, may appear transiently, usually in about thirty days and in the case of animals of five to seven millimeters shell length. Individuals which have reached a shell length of fourteen or fifteen millimeters after forty days culture are of a size where female development may be under way. The condition of the gonads in this material will be reported later when microscopic sections are made.

The effect of sulfhydryl inhibitors on the anaerobic glycolysis of scallop and thyone muscle. J. Harting.

Steinbach and Kawata (*Federation Proc.*, 3: 46; 1946) reported that iodoacetate accelerated the rate at which a cut surface of scallop muscle changed to restore the normal membrane conditions, as indicated by measurements of the injury potential. Since iodoacetic acid is known to inhibit metabolic processes by combining with the —SH groups essential for enzyme activity, it was decided to investigate the effect of the sulfhydryl inhibitors on the glycolysis of scallop muscle. The striated portion of the scallop muscle was teased into thin strips and suspended in van't Hoff's artificial sea water, containing 0.0238 M $NaHCO_3$, 0.01 M glucose, and saturated with $N_2:CO_2$ (95:5), pH 7.2. The rate of glycolysis was measured manometrically at 26° C. with $N_2:CO_2$ as the gas phase. When the alkylating agents, iodoacetate (0.001 M) and iodoacetamide (0.005 M), were added twenty minutes before manometric readings started, there was inhibition of glycolysis (CO_2 output) about forty minutes later. When they were added one hour after the measurement of glycolysis started, there was a steady increase in CO_2 output, which continued for one to two hours. In contrast to scallop muscle, the glycolysis of thyone muscle was inhibited by iodoacetate whether it was added before or during the measurement of glycolysis. Iodosobenzoate (0.001 M), an oxidizing agent, and p-chloromercuribenzoate (0.001 M), a mercaptide-forming compound, produced an immediate increase in the glycolysis of scallop muscle. The increase in CO_2 evolution in the case of these reagents was independent of the time at which they were added. p-chloromercuribenzoate (0.001 M) also had a stimulating effect on thyone muscle.

On the relation between oxygen consumption, fertilization membrane formation, and cell division in artificially fertilized Arbacia eggs. A. K. KELTCH AND G. H. A. CLOWES.

Oxygen consumption of sperm fertilized Arbacia eggs is about 2.7 times that of unfertilized eggs under present conditions of experiment. This investigation is to determine (1) whether the same ratio obtains for artificially fertilized eggs and (2) whether any such increase in oxygen consumption is associated with (a) formation of a fertilization membrane or (b) subsequent cell division, or both.

Artificial parthenogenesis was accomplished by exposing unfertilized Arbacia eggs for 25 minutes to sea water to which sodium chloride had been added to give a concentration equivalent to 1.05 M NaCl. The egg-containing hypertonic solution was then diluted with 30 volumes of sea water, the eggs allowed to settle, the supernatant fluid syphoned off, and the egg suspension once more diluted with 30 volumes of sea water, allowed to settle, decanted, and the eggs taken to a known volume of approximately 2 per cent. Equivalent concentrations of sperm fertilized eggs were subjected to the same series of procedures. Counts were made on both artificially and sperm fertilized egg suspensions, equivalent amounts were put in Warburg vessels for two

TABLE I

Oxygen consumption and per cent division of Arbacia eggs (A) sperm fertilized and (B) artificially fertilized

(From previous work the oxygen consumption of unfertilized Arbacia eggs is from 1.5 to 1.7 c. mm. O_2 per hour per 10 c. mm. eggs.)

Exp. No.	c. mm. O_2 per 10 c. mm. eggs per hr.		Per cent of eggs divided in 3½ hrs.	
	A	B	A	B
23 W	4.8	3.6	98	45
24 W	4.7	4.3	98	45
25 W	4.4	3.8	98	24
28 W	5.9	6.0	98	24
29 W	5.1	5.3	98	26
30 W	5.3	5.5	98	18
31 W	5.2	5.0	98	24
Average	5.1	4.8		

In all cases the eggs gave 98 to 100 per cent fertilization membranes.

hours to determine oxygen consumption, and samples were removed to determine extent of cell division.

The following table indicates that oxygen consumption was approximately the same for artificially and sperm fertilized eggs. This is extremely interesting in view of the fact that while in both the artificially and sperm fertilized eggs membrane formation was from 98 to 100 per cent, recognizable cell division never exceeded 45 per cent and was as low as 18 per cent in one set of artificially fertilized eggs. It appears, therefore, that the increase in oxygen consumption is associated with some change of condition of the eggs when the fertilization membrane has been formed and is not dependent on subsequent cell division.

Effect of dinitrocresol on oxygen consumption and cell division in artificially fertilized as compared with sperm fertilized Arbacia eggs. A. K. KELTCH, C. P. WALTERS, AND G. H. A. CLOWES.

Certain substituted phenols stimulate oxygen consumption of sperm fertilized Arbacia eggs and, at concentrations giving optimum oxygen consumption, reversibly block cell division. This investigation is to determine whether the same effect is exerted on oxygen consumption and division of artificially fertilized eggs.

Artificial parthenogenesis was accomplished as described in previous paper except that after 25 minutes in hypertonic solution sample egg suspensions were diluted twice with 30 volumes sea water or sea water containing desired concentrations dinitrocresol before bringing egg suspensions to 2 per cent concentration. Equivalent samples of sperm fertilized eggs were subjected to the same series of procedures. Egg counts were made and two-hour oxygen consumption determined for artificially and sperm fertilized series in sea water and various con-

TABLE I

Stimulation by 4,6-dinitro-o-cresol of oxygen consumption of Arbacia eggs fertilized by (A) sperm and (B) artificial parthenogenesis

The figures presented in the table represent values of the ratio:

$$\frac{\text{oxygen consumption of treated eggs}}{\text{oxygen consumption of control eggs}} \times 100.$$

Exp. No.		Molar concentration DNC $\times 10^{-6}$					
		0	4	8	16	32	64
A	23 W	100			253	172	134
	24 W	100			246	179	160
	25 W	100			236	151	119
	28 W	100			213	159	126
	29 W	100			182	152	134
	30 W	100	173	217	230		
	31 W	100	216	234	222		
	Average	100	194	225	226	163	135
B	23 W	100			201	175	
	24 W	100			266	214	178
	25 W	100			161	132	120
	28 W	100			197	144	109
	29 W	100			170	152	120
	30 W	100	155	199	195		
	31 W	100	200	227	206		
	Average	100	187	213	214	163	132

centrations of dinitrocresol, counts made to determine extent of division, and samples returned to sea water to ascertain extent of recovery.

The accompanying table shows that various concentrations of 4,6-dinitro-o-cresol gave approximately the same percentage increase of oxygen consumption in artificially and sperm fertilized eggs. Furthermore, the block to cell division occurred at the same concentrations of 4,6-dinitro-o-cresol and also of 2,4,6-trichlorophenol in artificially and sperm fertilized eggs. However, when returned to sea water after three hours' exposure to reagents, sperm fertilized eggs showed 100 per cent recovery in all concentrations, whereas artificially fertilized eggs exposed to higher concentrations developed only to an abnormal, many-cell stage.

Urea excretion in the smooth dogfish. RUDOLF T. KEMPTON AND BURTON STECKLER.

Study of urea reabsorption by the kidneys of the smooth dogfish has yielded preliminary data indicating the magnitude of the reabsorptive process. As is well known, the elasmobranchs utilize high levels of blood urea as a mechanism of keeping their blood isotonic with sea water. The reabsorption of urea is a fundamental part of the method of maintaining this high blood level.

Inulin clearance was used as a measure of glomerular filtration. Simultaneous urea clearance permits the calculation of the behavior of kidney in relation to urea as well as to water.

In the series of experiments the plasma level of urea (plus ammonia) nitrogen averaged 961 mgm.%, the equivalent in urea of more than 2 per cent. Plasma rather than whole blood determinations were made due to the impermeability to urea of the red cells of this species. Urine urea (plus ammonia) levels ranged from 24 to 73 per cent (average 48 per cent) of the plasma concentration. This fact gives clear indication that urea reabsorption is due to the activity of cells rather than to a passive back diffusion.

While from 27 to 74 per cent (average 60 per cent) of the water in the filtrate was being reabsorbed, from 72 to 92 per cent (average 82 per cent) of the filtered urea was reabsorbed. The data thus far do not indicate any clear correlation between the two processes.

Calculations of the amount of reabsorption per 100 ml. of filtrate yield a comparatively constant value, ranging from 710 mgm. to 922 mgm. (average 808 mgm.).

On the basis of these and earlier data on water reabsorption it is provisionally concluded that under the experimental conditions there is considerable variation in the number of active nephrons, both in different animals and in the same animal at different times. Further experiments are to be conducted to determine the limits of urea reabsorption and to determine whether this process is related to the reabsorption of other materials such as water and reducing sugar.

The transient response of luminous bacteria to sudden temperature increments and related experiments on drug action and protein denaturation. G. L. KREEZER AND E. H. KREEZER.

In a linear physical system, the determination of its transient response to a sudden disturbance supplies information about the internal organization of the system not obtainable from static measurements. The transient response refers to that portion of the response during which the system is changing from a preceding steady-state to that following the disturbance. The present experiments were undertaken to test the usefulness of such determinations of transient response in non-electrical biological systems. Suspensions of luminous bacteria (*Achromobacter fisherei*) were subjected to sudden increments of temperature, and the rate of temperature rise and the associated rise in the brightness of the suspension recorded with amplifier-oscillograph equipment. These records show a definite lag in the luminescent response behind temperature. Supplementary experiments and computations indicate that the lag cannot be attributed to a lag in heat transmission through the suspension, or through the bacterial cell membrane. It seems necessary to attribute it to a lag occurring in the chain of chemical reactions underlying bioluminescence. Various chemical hypotheses are considered.

Preliminary experiments were also performed on the transient decay of luminescence associated with the sudden application of various drugs (ethyl alcohol, urethane, sulphanilamide). Transient response curves were obtained with time constants of the order of fractions of a second.

The results thus suggest that this procedure may provide a useful method for measuring the rate of action of drugs on living cells.

In order to investigate similar phenomena in purely chemical systems, protein solutions were selected as offering suitable material. Preliminary experiments upon dilute solutions of serum albumin show that sudden increments of temperature produce an immediate and progressive increase in the turbidity of the solution, followed by apparently complete reversal on cooling. Further experiments on the "denaturation response curves" of different proteins, and the response to different denaturing agents are in progress. (These experiments were aided by a grant from the Penrose Fund of the American Philosophical Society.)

The effect of sinus gland extract on certain enzyme systems. ELOISE KUNTZ.

The endocrine function of the crustacean sinus gland has been well established by numerous studies of its diverse effects on intact animals. This is a preliminary report on the effect of sinus gland extract on enzyme systems.

Extracts were prepared by suspending the glands in water, crushing them and then freezing. Several hours before use the extract was thawed and stored in the refrigerator. The animals used were lobsters, *Homarus americanus,* and the spider crabs, *Libinia dubia* and *L. emarginata.*

The ability of tissue homogenates to utilize 2–6-dichlorophenol indophenol as a hydrogen carrier was measured and found to be no greater in the presence of sinus gland extract. One of the principal cellular constituents capable of reducing this dye is glutathione. The addition of an extract of sinus gland doubled the reducing ability of glutathione. Sinus gland extract alone did not reduce the dye. This suggested that the hormone may serve as a hydrogen carrier by reducing active groups such as sulfhydryls.

The coupled oxidation-phosphorylation by a homogenate of *Libinia* ventral nerve ring from eyestalkless animals has also been studied. In a system containing homogenate, adenylic acid, glutathione, arginine and inorganic phosphate the oxidation of Na succinate was used to supply the energy for the formation of arginine phosphate. This system binds inorganic phosphate in the presence of oxygen. The rate of phosphate uptake is enhanced by the presence of sinus gland extract. In the absence of glutathione or oxygen organic, phosphate is hydrolyzed to phosphoric acid. In this reaction, too, the addition of sinus gland extract accelerates the breakdown of phosphate. These experiments indicate that sinus gland extract acts as an accelerator in both cases, but it apparently does not affect the direction of the reaction.

Germ cell formation of Tubularia crocea with special reference to the germ plasm theory. C. K. LIU.

The formation of the male and female germ cells of the hydroid *Tubularia crocea* has been studied with a view to test the germ plasm theory. In contradiction to Weismann's hypothesis, the germ cells are of endodermal origin instead of ectodermal. No interstitial cells are involved in their formation, and there is neither early segregation nor migration of germ cell. The entocodon is formed by an apical proliferation and subsequent delamination of the endoderm of the gonophoral bud, which is nothing but an extension of the body wall of the hydranth. From the entocodon the germ mass is differentiated. The latter, however, has been found to develop not only into germ cells but also into scattered septum cells, which are not germinal, but somatic. The "germ plasm" therefore is originated from somatic cells and in turn gives rise to somatic cells in addition to germ cells. Regeneration experiments further indicate that the general somatic cells throughout the length of the stalk have the potentiality of forming functional germ cells, male or female. All these go to prove that the "germ plasm" and soma remain permanently mixed with each other in the stalk tissue, and such a distinction is far-fetched and meaningless. The result of this study is therefore incompatible with the germ plasm theory.

Observations on the hemolytic action of urea and sodium salicylate. LOIS H. LOVE, W. E. LOVE AND M. H. JACOBS.

It has been suggested by Wilbrandt that the primary action of certain hemolytic agents on erythrocytes is a denaturation of proteins of the cell surface, resulting in a permeability to cations followed by osmotic hemolysis, through the operation of the Donnan equilibrium. Observations were accordingly made on the hemolytic action of two agents known to be efficient protein denaturants, namely, urea in high concentrations and sodium salicylate. In general, both substances proved to be much more difficult to work with than butyl alcohol and sodium tautocholate, the behavior of which is discussed elsewhere. Part of the difficulty was due to the production of internal changes in the cells, which sometimes very obviously affected their osmotic properties; part of it was due to the smallness of the difference in the exposures required to produce permeability to Na ions, on the one hand, and to sucrose molecules, on the other. With sufficient patience, however, it was possible with both agents to prepare cells which showed several of the osmotic characteristics of cation-permeable erythrocytes already discussed, namely, (1) hemolysis in NaCl and preservation in sucrose solutions, with a marked protective action of sucrose in mixed solutions, (2) a minimum rate of hemolysis in NaCl solutions at a pH near the isoelectric point of hemoglobin, and (3) a strong and rapid shrinkage of cells treated in NaCl or a mixture of NaCl and sucrose on being placed in pure sucrose isosmotic with blood. The shrinkage in a sucrose solution of this concentration was several times observed to restore to visibility erythrocytes that had already swollen to the point of invisibility, thereby giving rise to a type of "reversible hemolysis" somewhat different from the common one in which shrinkage is produced by a sudden increase of the osmotic pressure of the external solution.

Effects of thiourea and phenylthiourea upon the development of Plethodon cinereus.
W. GARDNER LYNN.

The Red-backed Salamander is unusual among amphibians in that it lays large-yolked, terrestrial eggs and has no aquatic larval stage. During development certain larval characters, such as external gills, appear transiently but these degenerate before hatching and the young salamanders emerge from the egg with the adult body form. In view of the well-known importance of the thyroid hormone in the metamorphosis of the larvae of ordinary amphibians, a study of the rôle of the thyroid in the precocious metamorphosis of *Plethodon* was undertaken through the use of thyroid-inhibiting drugs.

Fifteen clutches of eggs were collected between July 2 and July 14 in rotting logs in the Devil's Lane Tract near Woods Hole. At the early limb-bud stage the jelly layers and vitelline membranes were removed and, for each clutch, some eggs were kept in tap-water while others were kept in 0.01 per cent thiourea or in 0.005 per cent phenylthiourea. Control animals developed normally and underwent gill resorption and other metamorphic changes on about the 25th day. Experimental animals in both solutions retained the gills, failed to shed the larval skin and showed no differentiation of eyelids as long as treatment was continued. Cessation of treatment was followed by metamorphosis; gill resorption beginning within three days. It appears that, in *Plethodon*, the later stages of intra-oval life constitute a metamorphosis which is under thyroid control. By inhibition of the thyroid the metamorphic changes can be prevented and an "aquatic larva" is thus produced in a salamander which normally has no such stage.

An additional effect of phenylthiourea was a depigmentation of the embryo involving both the skin and the pigmented coat of the eye so that animals kept in this drug were entirely without color. This effect is probably to be attributed to failure of melanin production through inhibition of tyrosinase.

Are —SH groups involved in the penetration of glycerol into human red cells?
A. K. PARPART, E. S. G. BARRON AND T. DEY.

The apparent rate of penetration of glycerol into human red cells, as studied by hemolysis, is delayed by a number of compounds that inhibit the action of —SH groups (parachlormercuricbenzoate; Hg; chlorpicrin; Cu). Other inhibitors have no such effect (paracarboxyphenylarsenoxide; Cd; iodosobenzoate; iodoacetamide). Volume change methods for the study of the penetration of glycerol did not show any inhibition, except in the case of Cu. It is suggested that those inhibitors which are active do so through their action on the outward diffusion of hemoglobin in the hemolysis studies. Human red cells exposed for 30 minutes to parachlormercuricbenzoate also show a delay for water and ethylene glycol hemolysis; a further confirmation of the above suggested mode of action.

A comparison of the energy requirements of the sand dollar and sea urchin eggs. W. A. Robbie.

With respect to maintenance and the process of cleavage, marine eggs may be classified into three groups: (1) those that demand respiratory energy for both maintenance and cleavage; (2) those that need oxygen for cleavage but will survive without it; and (3) those that will both divide and survive under anaerobic conditions. These metabolic characteristics of the developing egg may be studied by experiments at low oxygen tensions or by cyanide inhibition of the heavy metal enzyme systems responsible for respiration.

The sand dollar, *Echinarachnius parma,* is a good example of the type of egg that requires an aerobic system for both cell division and maintenance. Arbacia represents the second type, and Fundulus will survive and cleave in cyanide or in the low-oxygen bottom mud which is its usual environment. Experiments on Echinarachnius at the Mount Desert Island Biological Laboratory and on Arbacia at Woods Hole show that respiration and cell division are inhibited in both by low concentrations of cyanide. The striking difference is in the irreversibility of inhibition of respiration in the sand dollar egg, whether by oxygen lack or cyanide. One-half hour in one ten-thousandth molar cyanide results in eventual cytolysis of most of the eggs, whereas Arbacia will withstand 24 hours with no apparent ill effects. The coincidence of this cytolysis with the point of marginal inhibition of respiration by cyanide suggests that a metabolic factor is involved.

The difference in the behavior of the Echinarachnius and the Arbacia egg may be due to the activity of a glycolytic system, since it has been shown that Arbacia eggs in cyanide produce lactic acid. This glycolytic process perhaps provides enough energy to maintain the egg during the period of respiratory inhibition. Glycolysis measurements on Echinarachnius have not yet been made.

A new peritrich from Woods Hole. M. A. Rudzinska.

During the month of August, while making daily inspections of material from live cars immersed in the Eel Pond, I found a peritrichous ciliate not yet described. Observations were limited due to the fact that only three individuals were found.

These three ciliates, about 0.2 mm. long, were attached to a tiny smooth stick with transverse stripes. The body form is cup-like and narrower at the anterior and posterior ends. The anterior region is conspicuously ciliated with one ring of long cilia which surround a very large cytostome. The cilia are in continuous movement. There is one contractile vacuole located near the cytostome. The posterior end has two pseudopodia like lamellae by which it is attached to the stick. The body is not contractile.

These forms have a very unique movement along the stick. They go from one end of the stick to the other, stopping when they reach the end of the stick and then returning the whole length again. The form of the body does not change during this movement, nor do any contractions of the lamellae, by which they are attached, occur when they move. The motion is like a smooth, constant sliding. When removed from the stick they affix to the slip and stop moving. Only the cilia of the cytostome retain their movements.

The above described, very characteristic motion was also observed by Dr. A. A. Schaeffer, and Dr. W. D. Burbanck. It is interesting to note that the individuals seen by these men were attached to the same sort of stick that I have described.

It seems to me that the described peritrich belongs to the family Scyphididae Kahl because the posterior end is without stalk and they stick to objects by attaching lamellae. It is a new genus and a new species.

I only had this material for a short time, and did not make stained preparations. More details of the construction of the body will help to explain the unusual type of movement.

Androgenetic males and X-ray induced cytoplasmic injury. Anna R. Whiting.

Unlaid eggs of wild type *Habrobracon juglandis* females X-rayed in first meiotic metaphase (lethal dose 2,200 r), if unfertilized, develop into wild type haploid gynogenetic males; if fertilized by untreated sperm with recessive factors they develop into wild type diploid females or recessive haploid androgenetic males. Unlaid eggs X-rayed in first meiotic prophase (lethal dose 48,000 r) behave in the same manner except that no androgenetic males are produced. Androgenetic males appear to arise through retardation of the egg pronucleus by chromatin bridges, numerous after treatment of metaphase I. These bridges are formed but rarely after treatment of prophase I and, when present, are not numerous enough to retard female pronucleus. In spite of the extreme sensitivity of metaphase I eggs, androgenetic males develop in them when they are irradiated with doses up to lethal for prophase I, about twenty-two times their own lethal dose. At doses above this none has been produced. Their number decreases as the prophase I lethal dose is approached. This indicates cytoplasmic injury at high doses. Two factors, then, are involved in development of androgenetic males, (1) injury to the egg chromosomes favoring their production, (2) injury to the cytoplasm inhibiting it. The dose at which these two effects "cross," marked by highest production of androgenetic males, is about 12,000–15,000 r. At about 48,000 r cytoplasmic injury completely dominates the former.

Action of X-rays on mating types and conjugation of Paramecium bursaria.

RALPH WICHTERMAN.

Opposite mating types of the green *Paramecium, P. bursaria,* highly reactive for the mating reaction were irradiated in celluloid boats at dosages ranging from 100,000 to one million roentgens. Irradiation affects the mating reaction, destroys the zoöchlorellae and delays and lengthens conjugation.

When opposite mating types are irradiated up to 300,000 r and mixed, only small clumps of usually less than a dozen paramecia are formed instead of the large clumps of a hundred or more shown in the controls. The mating reaction does not result in the immediate formation of joined pairs in conjugation after irradiation. However on the day following irradiation, small clumps are formed again which then result in conjugating pairs for those irradiated up to 200,000 r; a second day must elapse for conjugation to occur in those irradiated with 300,000 r. When paramecia of one mating type are irradiated up to 300,000 r and mixed with unirradiated paramecia of the opposite mating type, clumping occurs similar to that of the controls but does not result in conjugation until the following day. When both mating types are irradiated with 400,000 r and 500,000 r respectively and mixed, the mating reaction is slower and the number of paramecia in clumps smaller. The mating reaction is temporarily stopped with 600,000 r, but small clumps are formed three days after irradiation. In these higher dosages, the conjugation lag increases; with mating types irradiated with 600,000 r, conjugating pairs are not formed until 12–14 days after irradiation although clumping occurs earlier.

Animals of opposite mating type when irradiated with 700,000 r and then mixed show no mating reaction but do so when mixed with unirradiated paramecia of opposite type. At this dosage and up to one million roentgens paramecia live for a time but do not survive. At 600,000 r and less, many paramecia not only survive but after a lag period have a fission rate similar to the controls.

A method is now available for obtaining colorless, zoöchlorellae-free races of *P. bursaria* which occur with rarity in nature. Dosages of 300,000 to 600,000 r destroyed the zoöchlorellae and when the irradiated specimens were isolated, new clones of zoöchlorellae-free paramecia were obtained. After two weeks, these clones of irradiated and colorless *P. bursaria* of a given sex type were mixed with typically green specimens of opposite sex type. Mating and conjugation followed. The sex type of irradiated zoöchlorellae-free paramecia was the same as before irradiation.

Unirradiated paramecia not only survive but reproduce in clone cultures which had been irradiated with one million and less roentgens, hence such fluid does not appear to be toxic to the organisms.

There is clear evidence of a difference in susceptibility to roentgen rays in the mating types tested.

Irradiated conjugants (300,000 r) remain together much longer in sexual union than those of the controls; a small percentage are unable to survive.

Dosages of 100,000 r stimulate activity of the paramecia but higher ones progressively retard locomotion, markedly increase the viscosity of the cytoplasm and produce small, clear vesicles (hyaloplasm) on the pellicle which then pass into the culture medium.

Effect of temperature on light absorption by the cell-free coelomic fluid in Phascolosoma gouldii. Charles G. Wilber and Ruth P. Alscher.

Very little is known concerning the physical characteristics of invertebrate body fluids. In an attempt to ascertain the relationship of invertebrate fluids to those of higher animals, a series of experiments was made to test the effect of heating on the absorption of light by the cell-free coelomic fluid in *Phascolosoma gouldii.* Coelomic fluid was removed from the worms, centrifuged, and the cell-free supernatant fluid sealed in carefully matched soft glass test tubes. Some of the tubes were heated for 5 minutes in a carefully controlled water-bath at temperatures from 24° C. to 90° C.; others were heated at similar temperatures for 20 minutes. The absorption of light by the heated fluids was compared with that of distilled water in an electric photometer. If the value recorded on the photometer is plotted against temperature, evidence of a decrease in light absorption at about 40° C. followed by an increase beginning at 50° C. is obtained. A graph obtained by plotting optical density against temperature indicates that the density decreases at about 40° C. but suddenly increases rapidly at about 50° C. Apparently, there are 2 critical temperatures for the cell-free fluid in *Phascolosoma:* (1) 40° C. above which heating brings about a decrease in particle size, with resulting decrease in light absorption; (2) 50° C. above which particle size is increased, with resulting increase in light absorption, possibly by intramolecular hydration. The consistent preliminary decrease in absorption of light between 40° and 50° C., which is found in the fluid of *Phascolosoma,* does not obtain in horse serum (see DuNouy, 1945, The critical temperature of serum).

Observations on the hemolytic action of sodium taurocholate. Marian Willis, W. E. Love and M. H. Jacobs.

The effects of hemolytic concentrations of sodium taurocholate on mammalian erythrocytes can be stopped short of hemolysis by suitable dilution with 0.3 M sucrose. Cells so treated can be kept with little change for hours. They show permeability to non-electrolytes that can be regulated within fairly wide limits by the concentration of taurocholate employed and to a lesser extent by the length of exposure. Too great an exposure may produce a permeability even to sucrose.

Taurocholate-treated cells give osmotic evidence in the following ways of an induced permeability to cations, the degree of which can also be varied by the character of the exposure: (1) immediate rapid shrinkage in pure sucrose solutions isosmotic with blood, (2) swelling and hemolysis in NaCl solutions of any strength, (3) a protective effect in NaCl solutions of low concentrations of sucrose, (4) shrinkage followed by recovery to more than the original volume on the addition of concentrated NaCl to cells in an appropriate mixture of NaCl and sucrose, and (5) a minimum rate of hemolysis of previously treated cells in NaCl solutions near the isoelectric point of hemoglobin.

While the swelling of cation-permeable cells is favored by increasing alkalinity above the isoelectric point of hemoglobin, as demanded by the Donnan principle, the production of a condition of cation-permeability by taurocholate falls off rapidly as the pH rises. The simultaneous existence of these two dissimilar types of action results in a somewhat complex overall pH effect. It also explains the observation of Ponder that according to the time of its addition alkali may either increase or decrease the rate of taurocholate hemolysis.

Native protein networks and biological morphology. Dorothy Wrinch.

A major problem of biological morphology is the explanation of the relationship between the characteristic gross morphology or "habit" of an organism and its atomic structure. In mineralogy there is a comparable passage from the world of angstroms and the atomic pattern of a crystal to the visible level and crystal "habit." A possible approach to the biological problem may be suggested according to which the parallel use by biologists and mineralogists of the term *habit* is not just a coincidence, but the key to the problem.

According to the native protein theory of the structure of cytoplasm, (1) the essential entity is a network of protein units of low connexity running throughout the organism, unicellular or multicellular, the plasma membrane of each cell being a localized "skin" of higher connexity;

and (2) the interlinkings of the protein units, with or without non-protein units as intermediates, are of the degree of precision achieved by intermolecular associations in crystals (Wrinch, *Cold Spring Harbor Sym.*, **9**: 218; 1941; *Collecting Net*, **17**: 83; 1942). We now suggest that the non-protein material secreted, *e.g.*, the successive siliceous shells of diatoms, and cellulose shells of desmids, the calcium carbonate shells of corals, are laid down on the plasma membranes with the same crystal-like precision, so that such structures may be viewed as intergrowths onto the underlying network of functional proteins, on which biological specificity depends, of essentially the same structural nature as the intergrowths of inorganic crystals (*e.g.*, quartz onto calcite).

The "symmetry" of living organisms discussed by biologists in the branching of trees and corals, phyllotaxis, etc., is not symmetry in the classical sense: the actual point symmetry in each such case is a totally inadequate description of the morphological situation. Rather is it of the type encountered in such complex intergrowths as the dendritic forms, frequently encountered with gold, copper and other species, especially those belonging to the *cubic* system (*Dana's Mineralogy*, 1922; p. 173). As is well known, twins and other mineral intergrowths are, in many cases, direct indications of a major atomic theme of high symmetry (Wrinch, *Am. Mineral*, in press). Correspondingly a diatom shell is, like a snowflake, a significant complex of intergrowths, not a single crystal. Its biological essence resides in its relation to the underlying atomic patterns of the proteins in the plasma membrane and a cubic skeleton for protein molecules (Wrinch, *Biol. Bull.*, **89**: 192; 1945) provides living matter with just such a major theme.

PAPERS READ BY TITLE

Permeability of eggs of Arbacia punctulata to radioactive phosphorus. Philip H. Abelson.

Studies have been made of the permeability of unfertilized and fertilized eggs using radioactive phosphorus. Eggs in batches of 100,000, obtained by the conventional techniques, were exposed to filtered sea water containing 5.8×10^{-3} mg. phosphorus in the form of Na_2HPO_4 per 100 cc. for varying periods of time. Aliquots of the eggs were washed four times under conditions which insured removal of all supernatant solution and freely diffusible phosphate. In the case of fertilized eggs, studies were made both at 10° C. and at 23° C. In addition the effect of a metabolic poison—4–6 dinitro-o-cresol—was observed. The generalized conclusion was that phosphorus take-up of the eggs was quite closely connected to cellular activity. Thus the amount of phosphorus taken up by the fertilized eggs was 40-fold greater than that of unfertilized eggs. It is interesting to note that oxygen metabolism has been found to differ by only a factor of three in this case. At 10° C. the amount of phosphorus found in the eggs was only one-seventh that found for a similar exposure at 23° C. As is well known the relative times for first cleavage are also about the same factor different. When 1.6×10^{-5} molar 4–6 dinitro-o-cresol is present oxygen metabolism is about doubled while cell division is almost stopped (Krahl, M. E., and G. H. A. Clowes, *Jour. Gen. Physiol.*, **23**: 413; 1940). Under these circumstances phosphorus take-up was diminished by a factor of six. A survey of the fate of the radioactive phosphorus taken in at 23° C. showed that most went into the "acid soluble fraction" (phosphorus compounds soluble in 5 per cent tri-chlor acetic acid). With short exposures to radioactive phosphorus the "acid soluble fraction" contained ten times as much activity as the "acid insoluble fraction." Most of the "acid soluble fraction" was in the form of organic phosphate. One interesting feature was that the phosphorus uptake did not increase immediately after fertilization. A transition period of seven to ten minutes elapsed in which a new level of phosphorus intake was attained. One hour after fertilization the amount of traced phosphorus per million eggs ($74\,\mu$ diameter) was 1.0×10^{-3} mg. The rate of increase was 2.0×10^{-3} mg. per million per hour. This rate remained constant during the next four hours.

Photosynthesis of Ulva. Mary Belle Allen.

The green marine alga *Ulva lactuca* has been found to possess properties which make it a highly desirable organism for studies of photosynthesis. Thallae can easily be washed free of possible bacterial contamination and cut into small pieces for manometric measurements. The maximum rate of photosynthesis in 0.1 M $KHCO_3$ in sea water is 2.5 mm.^{3} oxygen/min./γ nitrogen, as compared with 0.03 mm.^{3} oxygen/min./γ nitrogen under similar conditions for the algae commonly used in physiological studies, *Chlorella pyrenoidosa* and *Scenedesmus obliquus*. The respiration of freshly collected samples is less than one per cent of photosynthesis, while in *Chlorella* and *Scenedesmus* respiration is 5–10 per cent of photosynthesis. When *Ulva* is kept in the laboratory for several days, even in running sea water, the rate of photosynthesis drops and respiration increases. Fresh samples are extremely resistant to cyanide, photosynthesis being unaffected by 0.001 M KCN and only 60–75 per cent inhibited by 0.01 M KCN. Old samples, with lower rates of photosynthesis, are more cyanide sensitive.

When a sample of *Ulva* which has been illuminated is placed in the dark, evolution of oxygen and uptake of carbon dioxide continue. The rate of oxygen evolution and carbon dioxide absorption slowly falls off in the dark, the time required for decay depending on the rate of photosynthesis. Two hours is required for the gas exchange of a fresh sample to fall to zero in the dark, while with old samples the effect is over in 10–15 minutes. The photosynthetic quotient measured by the Warburg indirect method is 1.05 ± 0.06 during illumination and 0.98 ± 0.03 during the period of oxygen evolution and carbon dioxide uptake in the dark following illumination. The intermediates of photosynthesis in *Ulva* must therefore be much longer lived than those in most plants, perhaps because they are not as rapidly removed by respiratory reactions.

Chromoproteins in the tentacles of Physalia. ERIC G. BALL AND OCTAVIA COOPER.

In a study initiated to learn the nature of the toxic substance present in the stinging tentacles of Physalia several chromoproteins have been separated. The ground tentacles are extracted with water, centrifuged, and to the turbid bluish black supernatant ammonium sulfate is added to 35 per cent saturation. A heavy purplish-brown precipitate is centrifuged off. Further fractionation of this precipitate or characterization of its color has proved difficult. The bluish-green supernatant is brought to 60 per cent saturation with $(NH_4)_2SO_4$ and centrifuged. The colored precipitate is dissolved in water and dialyzed until salt free. A light blue precipitate forms, leaving a clear yellowish-green supernatant.

The blue precipitate can be dissolved in 0.9 per cent NaCl solution to give a slightly turbid solution with an absorption peak at 580–600 mμ. Acid ethyl alcohol added to the blue precipitate denatures the protein and yields a clear green solution with absorption maxima at λ 700 mμ and 370 mμ. Treatment of the blue protein precipitate with neutral ethyl alcohol denatures the protein but extracts very little color. Extraction of the precipitate now with 0.1 M phosphate buffer, pH 6.8, yields a clear blue solution of the prosthetic group with a well defined absorption peak at λ 585 mμ. This suggests that the blue form of the prosthetic group exists as a salt and that its combination with protein may involve a salt linkage. Tests for Cu and Ni gave negative results.

The greenish-yellow solution obtained after dialysis shows an absorption peak at λ 450 mμ All attempts to obtain its prosthetic group in solution free of its protein carrier lead to complete destruction of the color.

Solutions of both of these colored proteins exhibit lethal properties upon injection into Fundulus. Heat destroys these toxic properties. However, proof that the toxic action of the tentacles is due to these chromoproteins must await further work.

Effects of hydrogen ion concentration and of certain buffer systems upon the luminescent reaction of Cypridina luciferin and luciferase. AURIN M. CHASE.

Luciferin, extracted from the ostracod crustacean, *Cypridina hilgendorfii*, was carried through two cycles of purification by Anderson's procedure (*Jour. Gen. Physiol.*, **19**: 301; 1935. Luciferase, from *Cypridina*, was partially purified by dialysis.

The luminescent reaction, measured by Anderson's method (*Jour. Cell. Comp. Physiol.*, 3: 45; 1933), was studied from pH 5.5 to 8.7. Three buffer systems were used: KH_2PO_4-Na_2HPO_4, NaH_2PO_4-Na_2HPO_4 and KH_2PO_4-K_2HPO_4; all 0.067 M for phosphate and 0.01 M for sodium chloride. Attempts to extend the pH range with borate and barbiturate buffers were abandoned because the borate radically altered the luminescent reaction and the barbiturate abolished it.

Two distinct effects of pH were found, one upon the total light of the reaction and the other upon the first order reaction velocity constant.

Total light decreased with increasing pH, to a value at pH 8.7 about 20 per cent of that at pH 5.5 This effect was not due to non-luminescent oxidation of part of the luciferin during the reaction as in the case of temperature (Chase and Lorenz, *Jour. Cell. Comp. Physiol.*, **25**: 53; 1945), but apparently to a specific effect of pH upon the light-emitting system analogous to salt effects found by Anderson (*Jour. Amer. Chem. Soc.*, **59**: 2115; 1937).

The velocity constant of the reaction was maximal at about pH 7.2. It decreased to about 50 per cent of the maximal value at pH 8.7 and to about 15 per cent of the maximal value at pH 5.5.

At any given pH the velocity constant of the reaction (but *not* the total light) was affected by the sodium : potassium ratio of the phosphate buffer. For example, at pH 6.8 the velocity constant was measurably greater with the KH_2PO_4-K_2HPO_4 buffer or with the NaH_2PO_4-Na_2HPO_4 buffer than with the KH_2PO_4-Na_2HPO_4. This would seem to indicate some sort of sodium-potassium antagonism in this enzyme system.

Retardation of Arbacia egg cleavage by dinoflagellate-contaminated sea water (red tide). Ivor Cornman.

Two specimens of "red tide" were generously supplied by Dr. P. S. Galtsoff, Director of the U. S. Fisheries Laboratory, Woods Hole, Massachusetts.

An untreated decomposing sample of sea water taken from an area stained red by *Gymnodinium* when diluted 1 : 10 retarded *Arbacia* egg cleavage by one hour—100 per cent increase in cleavage time—if added 10 minutes after fertilization. When most of the H_2S was pulled off with a vacuum pump, the delay at 1 : 10 was only 3 minutes and at 1 : 5 was 15 minutes. Four days later all odor of H_2S was gone, but retardation was essentially the same, 9 per cent at 1 : 10 and 27 per cent at 1 : 5. Cytolysis resulted from exposure to 1 : 2. This inhibitory potency is equal to that of crude filtrate from some *Penicillium* cultures. This sea water sample killed *Fundulus* in 2 hours at 1 : 2 and in 5½ hours at 1 : 10. A "red tide" plankton sample suspended in sea water and preserved with $CHCl_3$ was evacuated until no odor of $CHCl_3$ remained. At 1 : 10 this retarded cleavage 10 minutes. *Fundulus* in this sample diluted with an equal part of sea water lost equilibrium and became sluggish in 2 hours, and in 6 hours at 1 : 10. They recovered motility in fresh sea water, but subsequently died. There appears to be some parallel between the toxicity to fish and to dividing eggs, but whether the same poison acts upon both and whether decomposition plays an important role remain to be determined. Studies conducted near the site with fresh samples of sea water and dinoflagellates should prove more helpful if uncontaminated test organisms are available.

Abnormalities of mitosis in tail-tips of Triturus torosus. Donald P. Costello and Catherine Henley.[1]

The material of this study consisted of 485 larvae developing from a batch of eggs of *Triturus torosus* shipped by express from Stanford University, California to Chapel Hill, North Carolina, in the usual type of thermos jug used for embryological materials. These larvae were raised under normal laboratory conditions. At the appropriate stage, the tails were clipped and prepared as whole mounts for cytological examination. Three additional successive clippings of the regenerated tails of the surviving larvae were performed at approximately 12-day intervals, during the period of daily feedings following yolk resorption, giving a total of 1,252 tail-tip preparations. The first regenerates are the best preparations. These (and others) show an unusually high percentage of cytological abnormalities of the types described by Alberti-Politzer (1923, 1924) after X-ray treatment of the corneal epithelium of *Salamandra maculosa*. The resting nuclei are of various sizes and forms, making it impossible to use nuclear size as a criterion of heteroploidy. Fragmentation of resting nuclei, and nuclear necrosis, with the production of numerous small basophilic areas, are among the causes of variation

[1] Aided by a grant from the Carnegie Research Fund of the University of North Carolina.

in nuclear size. Numerous mitotic abnormalities were also observed. These include: multipolar spindles; spindles from which chromosomes have been lost; lagging chromosomes and lagging or lost chromosome fragments at anaphase; bridges between anaphase or telophase chromosome groups, or between daughter nuclei; basophilic granules (chromatin?) outside spindle, etc. The chromosomal condition of these tail-tips includes euploidy and aneuploidy, a remarkable number showing mosaic areas. A preliminary survey of these mosaics reveals at least the following types: haplo-diploid; hypodiplo-diploid; hyperdiplo-diploid; diplo-triploid; diplo-tetraploid; diplo-pentaploid; diplo-hexaploid; diplo-octoploid.

Before attributing these cytological anomalies to any specific cause it is necessary to make a survey of natural heteroploidy in material collected and fixed in California.

A new form of the hydroid Stylactis. SEARS CROWELL.

Stylactis arge Clarke and *S. hooperi* Sigerfoos have not been critically compared so there is doubt as to their specific distinctness (for references see C. M. Frazer, Hydroids of the Atlantic Coast of North America). In late July this year Mr. Milton Gray collected and brought me a specimen of *Stylactis,* found growing on a Mytilus shell at Woods Hole. I have compared it with specimens on shells of *Nassa* (*Ilyanassa*) *obsoleta*.

In the following respects it differs from the typical *S. hooperi* as observed on *Nassa:* (1) Spines are absent. (2) The tentacles may be visualized as ". . . arranged in two verticils . . . those of the lower circlet sometimes shorter than those of the upper one," as described for *S. arge* by Clarke. (3) Gonophores are not directly below the tentacles but about one-third the distance from tentacles to base, as indicated in Clarke's figures of *S. arge*. (4) Tentaculozooids, not hitherto reported in the genus, are scattered among the other hydranths. These zooids are not enlarged terminally, not capable of forming a spiral, and not so long, when extended, as the largest gastrozooids.

The specimen differs from *S. arge* of Clarke in having shorter hydranths than his illustrations indicate, and more tentacles than he states are present. However, his description is equivocal on both points.

The specimen may be interpreted in one of three ways: (1) It may be *S. arge*. (2) It may be a new species. (3) It may illustrate the variation in a single species, as is likely true of *Stylactis arge* and *S. hooperi*.

The effect of X-rays on chromosomes and nucleoli in ovarian eggs of the salamander, Triturus pyrrhogaster. WILLIAM R. DURYEE.

The giant chromosomes of *Triturus pyrrhogaster* ovarian eggs react to X-irradiation in the same way as previously determined for those of the frog.

Four criteria of X-ray damage have been found. These are: (a) fragmentation of the chromonemata, (b) progressive disintegration of the lateral chromomere loops, (c) colloidal changes in nucleoli, and (d) gel → sol transformations in the central nucleoplasmic *chromosome frame*. High intensity radiation at the approximate rate of 6,120 r/min. was employed in dosages graded from 10,000 to 70,000 r, with the majority of the experiments at the 60,000 r level. Whole eggs were exposed in ordinary Amphibian Ringer buffered to pH 7.6, while isolated nuclei and and nuclear-free brei were irradiated in Ca-free N-medium (NaCl 0.66 g., KCl 0.014 g., dist. H_2O 100 cc.).

Multiple fragmentation of chromosomes appeared immediately following as little as 30,000 r. With 60,000 r or more 30–50 chromosome fragments could be found in each nucleus. The average fragment was of the order of 45–50 $m\mu$ in length, as compared with normal chromosomes 180–700 $m\mu$ long. Within these dosage limits, degree of fragmentation was roughly proportional to the amount of radiation. Some changes in lateral loops could be detected with as little as 10,000 r. By 30,000 r nearly all lateral loops had been stripped from the chromonemata, leaving the 13 chromosome pairs appearing as granular cylinders. Following exposures to greater dosages (30,000 to 60,000 r) large numbers of loop fragments were observed throughout the nucleoplasm surrounding the chromosomes. Above 60,000 r nearly all loop fragments had dissolved into numerous granules or micronucleoli (0.5–2.0 $m\mu$ diameter).

Nucleolar changes were strikingly evident. While normal Stage 4 nucleoli were clear polygonal or irregularly oval bodies, irradiated nucleoli had large vacuoles and granular interiors. Irradiation increased the normal average size from 15–20 mμ diameters to 30–45 mμ. Following the higher dosages clumping of nucleoli into masses over 150 mμ in diameter was frequent.

Changes in colloidal condition of chromosome frame substance were recognized by sinking and displacement of chromosome pairs. Solvation of the supporting gel usually required at least 50,000 r.

Eight sets of isolated nuclei were irradiated directly with 60,000 r. One set was given 100,000 r. In contrast to effects obtained with whole cells, all chromosomes were found normal in length and no evidence of fragmentation could be detected. Even with the highest dosage lateral loops were only partially dissolved. Nucleoli occasionally appeared vacuolated, clumped and with surface markings. Solvation of chromosome frame substance occurred in 80 per cent of directly irradiated nuclei.

Four other permutations of these conditions were examined: (*a*) non-irradiated nuclei exposed to normal cell brei, (*b*) non-irradiated nuclei exposed to X-rayed cell brei, (*c*) exposure of normal eggs to irradiated Ringer solution, and (*d*) non-irradiated nuclei exposed to alkalinized normal Amphibian Ringer, pH 8.0. In (*a*) no marked changes occurred until after 12 hours when slight vacuolization of nucleoli and some lateral loop damage was found. Irradiated brei, on the contrary, in less than 1 hour dissolved the lateral loops, vacuolated the nucleoli and displaced the chromosomes, but did not cause fragmentation. Irradiated Ringer produced no significant changes in whole cells. However alkalinized Ringer alone hydrolyzed lateral loops, caused nucleolar vacuoles and solvated the chromosome frame.

It is concluded that high dosages of X-irradiation will cause immediate fragmentation of egg chromosomes, loss of lateral chromomere loops and vacuolization of nucleoli when the nucleus is in the cell. On the other hand isolated nuclei and chromosomes do not react markedly when irradiated directly. Intermediate degrees of nuclear damage between the above extremes may be produced by exposure of non-irradiated nuclei to X-rayed cytoplasm or simply to alkalinized Ringer. These data are consistent with a hypothesis that X-ray damage to nuclear components is not primarily a direct effect, but an indirect one, probably caused by chemical changes in the cytoplasm.

The chromosome number of Triturus torosus. CATHERINE HENLEY AND DONALD P. COSTELLO.[1]

A total of 1,497 permanent whole-mount tail-tip preparations were made of the original and regenerating tails of 763 control and 14 experimental (cold-treated) *Triturus torosus* larvae. These included 244 larvae fixed in California and 533 shipped by express to Chapel Hill. A preliminary survey of the mitotic figures on these preparations has been made, and 126 metaphase chromosome groups studied and drawn. Many of these figures are from larvae with apparently normal diploid tail-tips throughout (criteria: nuclear size and normal mitotic figures), others from tail-tips showing obvious mitotic abnormalities (see abstract by Costello and Henley).

Since the chromosome number of this species has not been recorded, it is of interest to present the data on the 126 chromosome counts made to date. Six metaphases of four individuals which were obviously haploid showed 11 chromosomes. The remaining individuals included larvae which were diploid, triploid and mosaic heteroploids. Sixty-four of the metaphase figures from these had 22 chromosomes; eleven, 21 chromosomes; nine, 23 chromosomes; ten, 24 chromosomes. In five triploid figures, 30 or 31 chromosomes could be counted. In tetraploid and pentaploid figures, 42 and 51 chromosomes, respectively, were seen. The remaining counts varied from 15 to 27 chromosomes.

In crowded figures, chromosome counts are likely to be too low rather than too high. Since we frequently observed indications of chromosome elimination and lagging at anaphase in the abnormal tail-tips, it is likely that many of the figures examined were hypo- or hyperdiploid. In general, the evidence is in accordance with the conclusion that the diploid chromosome number of *Triturus torosus* is 22.

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[1] Aided by grants-in-aid from the Carnegie and Smith Research Funds of the University of North Carolina.

Polyphyodonty in the dogfish, Mustelus canis. JOHN D. IFFT AND DONALD J. ZINN.

The commonly accepted theory of tooth replacement in sharks describes them as moving forward from behind to replace those lost in front. This theory has been denied in recent years by Cawston. Since no direct observations of replacement apparently exist it was thought to be of some interest to determine experimentally the manner of replacement.

In one group of smooth dogfish two rows of teeth in the center section were extracted. All of the teeth back of the extracted area and all of the lateral teeth, except for the remaining ones of the first two rows, were marked with silver nitrate. In twenty-three days in some of the animals all of the teeth were replaced; in others only one row was replaced. The replaced teeth, as well as the rows lateral to them, had silver nitrate markings indicating that all of the teeth had rolled forward. Sections show that tooth buds are produced only in back of the entire erupted tooth area and not in any other location.

In another group the tooth-bud area was cauterized and as a result tooth replacement was inhibited. In others, triangular areas of teeth were extracted and replacement occurred without disruption of the normal arrangement of the teeth. All of the evidence seems to indicate that the original polyphyodont theory of tooth replacement is correct. The rate of replacement is apparently variable.

A method to immobilize fish for cardiac and other experiments with procaine. BRUNO KISCH.

The well known difficulty of performing cardiac experiments on fish was overcome with the following method of a preparation of the immobilized animal, practical without any loss of blood.

The animal is placed on its stomach. With a syringe and a 20 gauge medical needle for intravenous injections the skull is pierced somewhat caudally of the middle of a line connecting both eyes. According to the size of the fish ¼ up to 1 cc. 5 per cent solution of Novocain (Procaine hydrochloride) is injected intracranially and the needle removed.

The fish is immobilized and apparently also anesthetized satisfactorily within a few minutes and the effect lasts from ½ to 1 hour. A 1 per cent solution has no satisfying result but produces especially in dogfish with surety a long lasting nystagmus. A 10 per cent solution is dangerous because it more often kills the animal. Between 3 and 7 per cent is the most favorable concentration. The animal continues to breathe and if the head is submerged in a small seawater tank the heart can be used for a long time for experiments. If the fish is kept without water the heart beats also for a long time well. Intracranial injection of the same amount of saline or hypertonic saline solution has no similar effect.

The usual fenestration of the chest has to avoid lesions of the vessels to avoid loss of blood.

If the animal narcotised in this way (especially dogfish) is not operated on but brought back into the seawater tank it shows long lasting undulatory movements of its body, does not react to stimuli but recovers after about 1 hour completely. Whether the procaine during this time is reabsorbed or destroyed by a procaine esterase could not yet be ascertained.

This method will be useful for other experiments too; for instance, for metabolic investigations on the muscle or electric organ.

Cardiographic studies on the heart of fish. BRUNO KISCH.

Different species of fish (Selachiens and Teleostei) were investigated electrographically with so-called unipolar leads, the exploring electrode being placed on the surface of the heart or inside of auricle or ventricle. In spite of fish having only one auricle and one ventricle their cardiograms are of the same type as the ones of mammals and man. The asynchronism of excitation of the different parts of the ventricle makes for the complicated form of the fish electrocardiogram. It could be proven that the part of the ventricle first activated is its upper dorsal plane and mostly the left side of the base. Always the base of the ventricle is earlier activated than the apex. The endocardial surface is not activated earlier than the earliest activated parts of the surface of the ventricle but synchronously, in some instances even later.

The type of cardiograms registered from the inside of the ventricle depends to a high degree on the part where the electrode is placed. In Selachiens there is a clear differentiation between the endocardial electrogram of the right and of the left inside of the ventricle. The endocardial electrogram depends, of course, also on the type of contraction of the ventricle (partial systoles, normal systoles, extrasystoles, fractionated systoles).

The electrogram of the auricle is normally polyphasic showing a quick and a slow group of deflections. Sometimes it is bifid due to the asynchronous contraction of the different parts of the big auricle and its different appendages.

It was proven for the first time that the muscular bulbus cordis of Selachiens shows not only contractibility but also a high degree of automatism since in the exhausted heart of *Raja erinacea*, for instance, the bulbus is sometimes the pacemaker of the heart beat. This fact was also registered by means of electrocardiography.

Alkaline phosphatase activity in oöcytes of various marine invertebrates. E. J. KRUGELIS.

Using the cytochemical technique of Gomori, alkaline phosphatase activity was detected in the oöcytes of several marine invertebrates, including Arbacia, Asterias, Cynthis, Chaetopterus, and Mactra. This activity is present when sodium glycerophosphate, adenylic acid, or yeast nucleic acid are used as substrates for the enzyme. The cytoplasm of the oöcytes contain very little detectable enzyme activity while the germinal vesicle including nuclear sap and nucleoli shows a definite reaction in each form investigated. Basophilia is found in the cytoplasm and the nucleoli, while the germinal vesicle is faintly basophilic. Thus cytoplasmic basophilia and phosphatase activity seem independent in location in the oöcyte stages. Both basophilia and phosphatase activity occur together in all nucleoli except those of Mactra. This form possesses an amphinucleolus which includes a small basophilic nucleolus and a large oxyphilic nucleolus. Only the large oxyphilic nucleolus contains the enzyme activity. Thus, in the case of Mactra at least, basophilia seems completely separate from the location of enzyme activity.

Alkaline phosphatase activity in developmental stages of Arbacia. E. J. KRUGELIS.

The occurrence of alkaline phosphatase activity in Arbacia has been detected by use of the Gomori cytochemical technique. In the oöcytes, enzyme activity was detected only in the germinal vesicle. During this stage the vesicle stains with acid dyes such as fast green, but stains very faintly with the basic dye, toluidine blue or with Feulgen. The cytoplasm of the oöcyte, however, is strongly basophilic with toluidine blue. This basophilia of the cytoplasm is interpreted on the basis of Brachet's observations (*Embryologie Chimique,* Liège, 1945) as ribose nucleic acid. In the unfertilized mature egg, the fertilized egg, and early cleavage stages, the cytoplasm is the site of both basophilia and enzyme activity. Only sodium glycerophosphate was used as the enzyme substrate for these later stages. During blastula, gastrula, and plutei stages the cytoplasmic enzyme reaction seems to decrease parallel to the decrease in cytoplasmic basophilia. The nuclear enzyme activity increases in these stages, parallel to an increase in nuclear basophilia and Feulgen reaction.

This suggests that the enzyme and ribose nucleic acid have independent locations during oögenesis. During maturation the enzyme may be liberated into the cytoplasm and then parallels ribose nucleic acid content in the cytoplasm. This parallel location of the enzyme activity and nuclei acid is similar to the condition found in the late cleavage stages of amphibian embryo and suggests a possible active association during development.

A comparative study of iron content in marine invertebrates. SISTER ELIZABETH SETON MACDONALD AND CHARLES G. WILBER.

It is well known that sea water ordinarily contains only minute traces of iron in solution (*see* Cooper, *Proc. Roy. Soc. London* (*B*), **118**: 419; 1935). Many invertebrates have great power of concentrating iron in the tissues, but there is a lack of quantitative information concerning the amount of iron in the body fluids of various marine invertebrates. A series of colorimetric analyses were, consequently made to ascertain the iron content of the body fluids in several invertebrate animals. The amounts of iron (mean value of 10 animals unless otherwise indicated by number in parentheses after the species name) in mg./100 cc. fluid are as follows: *Phascolosoma gouldii*, 21.1; *Amphitrite ornata*, 9.8; *Glycera americana* (8), 15.5; *Nereis pelagica* (6), 9.6; *Chaetopterus variopedatus*, 3.1; *Venus mercenaria* (5), 2.0; *Asterias forbesi* (2), 2.8. The amount of iron is much higher in the annelids studied than in the mollusc or echinoderm. Such would be expected in view of the presence of heme pigments in the fluids of the former but not of the latter. It is interesting to observe, however, that there are consistently measurable amounts of iron in the mollusc and starfish, indicating that iron is in some way necessary for the general metabolism of these forms as well as for the annelids. A study of the variation in iron content in the above animals throughout the year is now in progress.

Fertilization studies on a non-conjugating race of Paramecium aurelia. CHARLES B. METZ AND MARY T. FOLEY.[1]

Metz (*Jour. Exp. Zool.*, **105**: 115; 1947) has shown that mixture of formalin-killed and living *Paramecium aurelia* of opposite mating types results in (1) specific clumping between living and dead animals, (2) induction of pseudo selfing and (3) activation (macronuclear breakdown and presumably other conjugation effects) of the living animals. In an attempt to discover the initiating mechanisms of activation and pseudo selfing, much of the previous work has been repeated on an abnormal (CM) race obtained through the kindness of T. M. Sonneborn. These CM animals give normal mating reactions but fail to conjugate.

It is found that formalin-killed CM animals induce pseudo selfing in living normal animals. However, living CM animals failed to self when treated with formalin-killed normal animals although the CM animals clumped strongly and specifically with these dead animals. From these results it seems highly unlikely that pseudo selfing involves transfer of mating substance from dead to living animals (as previously suggested). It is now believed that pseudo selfing depends upon interaction of specific "holdfast" substances which appear when the animal is activated but which are not mating type specific.

Formalin-killed CM animals can also induce macronuclear breakdown in single isolated living normal animals. From this it follows that the CM animals possess the activation initiating mechanism. However, living CM animals cannot be activated by treatment with dead normal animals. Therefore a block must prevent activation from proceeding much beyond the initial step in the CM animals. This evidence strongly favors the view that interaction of mating type substances initiates activation in *P. aurelia*.

Since the CM animals undergo natural autogamy, it follows that natural autogamy is not initiated through the same mechanism that operates in conjugation. An analogous situation may obtain in parthenogenesis, namely that the activation initiating mechanism of normal fertilization is by-passed in parthenogenesis.

Thecal morphology of some dinoflagellates of Woods Hole, with special reference to the "ventral area." DASHU NIE.

A total of eighteen species belonging to five genera was subjected to this study. *Exuviaella almonda* sp. nov., *E. marina*, *Prorocentrum schilleri* and *P. micans*, which represent all the Prorocentridae examined in this study, were revealed to have the same architecture. They have besides the two prominent bivalve-like plates four small platelets, lodging in the concavity of the left plate at the anterior end. Of these, three form the flagellar pore or pores, and one forms the spinose platelet. The number and disposition of these platelets are reported here for the first time.

The entity of the "ventral area" or sulcus of the genus *Diplopsalis* as well as the genus *Peridinium* has been found to consist invariably of six plates, namely, left and right anterior plates, left and right flagellar pore plates, the connecting plate and the posterior plate. These plates have been isolated in the following species: *Diplopsalis lenticula* f. *minor*, *D. asym-*

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[1] Aided by a grant from the National Institute of Health, U. S. Public Health Service.

metrica, Peridinium pallidium, P. globulus var. *quarnerense, P. marielebourae, P. conicum, P. claudicans, P. oceanicum, P. leonis* and *P. depressum.*

Four species of the genus *Goniaulax,* namely, *G. diegensis, G. digitale, G. spinifera* and *G. monocantha,* have been examined, and the result turned out to be quite different from that of Kofoid's (1911). A new plate formula is suggested: 3′, 3a, 6″, 6, 5‴, 2⁗. + 6 v.a.p. The reason for such a change is discussed.

Plastid development in the scutellum of Triticum sativum and Secale cereale. JOHN A. O'BRIEN, JR.

During germination of grains of *Triticum* and *Secale,* there are present in cells of the epithelium and the parenchyma of the scutellum, characteristic solitary aggregations of cytoplasmic elements which are preserved by mitochondrial fixatives. Each element at one stage is composed of a swollen head portion and an attenuated tail. They are so assembled that the heads are directed to the periphery and the tails toward the center of the group. The aggregation occurs in a differentiated region of the cytoplasm which, when preserved by certain fixatives, appears optically homogeneous and is thus set off from the granular cytoplasm. Usually the aggregation lies immediately adjacent to the nucleus. The elements comprising the aggregation eventually become dispersed in the granular cytoplasm and can be traced through several stages of dispersion. As the aggregation first begins to break up, the tail portion of the individual element develops a small knob at its distal end and the head portion diminishes slightly in size. After dispersion, the elements assume the characteristic form of plastids in fixed cells and vesicles which develop in them can be demonstrated to be starch grains. In some groups, the formation of starch grains occurs even before dispersion.

W. J. V. OSTERHOUT. No abstract submitted.

Vitamin A and lipid metabolism in the frog. CHARLES G. WILBER AND MARY E. F. TWOMEY.

Very little is known concerning the effect of vitamins on lipid metabolism in poikilothermal vertebrates. Consequently an investigation was undertaken to ascertain the effect of vitamin A on lipid metabolism in the frog. Frogs were fed corn oil in capsules and corn oil plus vitamin A in capsules respectively. After 48 hours the livers and kidneys were removed and analyzed for phospholipid, cholesterol, and fatty acid. The results were compared with those obtained from the analysis of unfed frogs used as controls. It was found that there was an increase of cholesterol, fatty acid, and total lipid in the liver and kidney of frogs fed corn oil alone but that there was a decrease in phospholipid in the same organs. Frogs fed fat plus vitamin A, on the other hand, showed an increase in phospholipid and a decrease in other lipids in the kidney and liver. The results clearly indicate that a high fat diet in the frog brings about a marked increase in the cholesterol and fatty acid in both liver and kidney. It is, moreover, evident that vitamin A stimulates to a marked degree the use of fat in the frog. These results are in accord with those obtained by other investigators using homoiothermal vertebrates (see Monaghan, *J. Biol. Chem.,* **98**: 21; 1932; Asada, *Biochem. Zeitschr.,* **142**: 44; 1923 and **144**: 203; 1924). The present investigation indicates that in the frog diet vitamin A is an essential component. On a diet lacking the vitamin there is an accumulation of lipids in the liver and a decrease in the synthesis of phospholipid by that organ.

Alkaline phosphatase in the early development of Fundulus heteroclitus. ANITA ZORZOLI.

A study of the localization of alkaline phosphatase in the development of *Fundulus heteroclitus* from late cleavage to the post hatching period when the yolk has been completely absorbed was carried out using the histochemical technique of Gomori. Each specimen was sectioned serially at 6 μ, cut into 2 section pieces and mounted on separate slides so that adjacent sections could be used for routine staining and for controls. Incubation was carried on for 12 and 24 hours at room temperature (approx. 26° C.) using Na β-glycerophosphate as substrate for the reaction.

There is a conspicuous lack of phosphatase activity in the large cells of the late cleavage and blastula stages as well as in the smaller cells of the gastrula stages. The first positive reactions occur at the beginning of differentiation of the nervous system. At this stage and shortly after, the nuclei of the neural cells stain deep brown-black. The cytoplasm is also positive but less strongly so. Outer ectoderm, endoderm and mesoderm cells stain uniformly positive and slightly less intensely than the nervous system. The large notochordal cells show the weakest reaction.

As tissue and organ differentiation proceeds, new regions showing strong activity appear while others decrease in activity. For example, by the time the cerebral hemispheres have formed there is a difference in intensity of reaction within the central nervous system itself, the anterior regions being more strongly positive than the posterior regions. Within the spinal cord there is a distinctly stronger reaction in both nuclei and cytoplasm of the dorsal third as compared with the more ventral regions.

Increase in phosphatase activity with increase in differentiation is shown in the gut. Both nuclei and cytoplasm stain more deeply at the time of hatching as compared with earlier stages and in addition there is developed an intense blackening at the proximal surface of the mucosal cells. This localization is reminiscent of the type seen in the small intestine of many mammalian forms.

PROGRAM AND ABSTRACTS OF SEMINAR PAPERS PRESENTED AT THE MARINE BIOLOGICAL LABORATORY, SUMMER OF 1947

JULY 15

Effects of sodium azide on the rate of amphibian development. THOMAS S. HALL AND FLORENCE MOOG.

In the course of an investigation of the effects of sodium azide upon the developing egg of the grass frog, *R. pipiens,* it was observed that exposure to this substance can retard or suppress development for considerable periods without preventing the possibility of normal development after termination of treatment. This response is being studied experimentally with a view to determining (a) the exact conditions under which it may be expected (results reported here) and (b) physiological factors involved (experiments in progress). The first phase of this investigation has brought to light three principal facts. (1) Under exposure to solutions of sodium azide (in 10 per cent amphibian Ringer's solution at *ca.* pH 7.2), varying in concentration from 7×10^{-6} to 5×10^{-5} M, early stages (cleavage to late neurula) undergo reduction in the rate of their development. It proved possible to continue exposure for 2 to 4 days, depending upon the concentration, without precluding the possibility of normal progress after removal from azide solution. Such treatment reduced the rate to less than half of normal. (2) Even where treatments were sufficiently severe (solutions stronger than 10^{-4} M) to induce complete arrest shortly after the treatment began, further development could occur. Individuals held at the gastrula stage for 48 hours went on to develop with every appearance of normality after treatment was stopped. In special instances, development was suspended for periods up to 86 hours; in these cases, however, not all individuals so treated developed normally, the incidence of abnormality increasing with the duration of the exposure. (3) If the organism was first exposed to solutions strong enough to cause complete arrest (e.g., 2×10^{-4} to 5×10^{-3} M, for 3 to 12 hours), it was found to be subsequently more sensitive to weak azide solutions than were previously untreated individuals. The mechanisms responsible for the foregoing results are under investigation.

Nucleic acids as growth factors in Drosophila. CLAUDE A. VILLEE AND HAROLD B. BISSELL.

Drosophila larvae of wild, vestigial, Bar, Double Bar, dachsous, four-jointed, aristapedia and tetraltera stocks were grown on sterile, chemically defined media containing salts, agar, amino acids, cholesterol, lecithin, ribose nucleic acid and sucrose in which ribonucleic acid was replaced by nucleotides, or purines and pyrimidines, singly or in combination. Larvae grew as well on nucleotides as on ribonucleic acid; the growth-promoting effect of ribonucleic acid resides not in RNA as such but in the purine and pyrimidine constituents of RNA, and especially in adenine. Guanine seems to have some function in regulating the growth stimulating action of adenine. Desoxyribonucleic acid inhibited growth and pupation of the larvae. The vestigial stock, in contrast to wild and Double Bar, was able to survive in the presence of benzimidazole, a structural analogue of the purines, and seems to have some biochemical mutant which enables it to utilize benzimidazole in place of the purines. One phenocopy was observed in the course of the experiments: a Double Bar larva grown on a medium lacking sucrose and nucleic acid developed into a fly whose eyes were large and oval-shaped, approaching the wild phenotype. The flies hatching out of larvae grown on experimental media were fertile and in many tubes a second generation of flies was raised.

ANNA R. WHITING. No abstract submitted.

July 22

The effects of podophyllin on the maturation and cleavage of the starfish egg. Ivor Cornman.

In a podophyllin concentration of 100 mg./L, breakdown of the germinal vesicle, penetration by the sperm, lifting of the fertilization membrane, and completion of cleavage by eggs in anaphase are to all appearances normal. On the contrary, the meiotic or mitotic spindle is quickly inactivated at 1 mg./L. The chromosomes released by spindle destruction form karyomeres which can engage in pseudomitotic cycles. If dosage is begun in the germinal vesicle stage, the released chromosomes or their karyomeres are unable to migrate to the periphery, but show evidence of responding to the meiotic rhythm by going through at least one cycle involving dissolution, chromosome formation, and reformation of the vesicular karyomeres. If the egg is fertilized or artificially activated, it goes through the same cycle. The karyomeres grow and coalesce, filling about half the volume of the egg by 24 hours. Occasionally they fuse to form a single nucleus which at 11 hours is $5/6$ the diameter of the germinal vesicle and contains 1 to 3 nucleoli. Up to 30 karyomeres have been counted ($2n = 36$), and up to 18 in eggs blocked just after completion of the second polar body. Variation in size permits the interpretation that some karyomeres contain more than one chromosome. The karyomeres persist as discrete bodies following disintegration of the egg, showing that they possess membranes and are not mere differentiated areas of cytoplasm. In 12 hours (or sooner if the eggs have been washed free of podophyllin or if they have been fertilized) there is irregular division showing no relation to the distribution of karyomeres. In the living eggs there sometimes appear to be asters participating in this division, but sections have not yet been studied to determine if true cleavage processes or merely pathological fragmentation are involved. Threshold doses (*ca.* 0.2 mg./L) which inhibit the early cleavages permit proliferation of the asters and eventually simultaneous division of the eggs into 16–32 cells, which subsequently divide to produce a normal blastula.

Preparation of a soluble cytochrome oxidase. W. Wainio, S. Cooperstein, S. Kollen, and B. Eichel.

Cytochrome oxidase occupies a key position in the chain of enzyme reactions that catalyzes the dehydrogenation of substrates, because the oxidase apparently is the enzyme that "activates" molecular oxygen. Much work has been done in an attempt to free the enzyme from the complex of which it is a part in order that it might be more fully characterized. All attempts, with the possible exception of a partially successful attempt by Haas (*Jour. Biol. Chem.*, **148**: 481; 1943), have failed to yield a soluble preparation.

The work of Hopkins, Lutwak-Mann, and Morgan (*Nature*, **143**: 556; 1939) and Keilin and Hartree (*Proc. Roy. Soc. London, B*, **127**: 167; 1939) suggested to us the use of bile salts to dissolve the cytochrome oxidase complex. Our results show that by extracting Keilin and Hartree's oxidase preparation (*Proc. Roy. Soc. London, B*, **125**: 171; 1938) with 4 per cent sodium desoxycholate, it is possible to obtain a preparation which resists centrifugation for 3 hours at 20,000 x g and which is clear to the naked eye. Such a preparation is 2.5 times as active as the original Keilin and Hartree suspension. A partial purification has been effected by fractionating with 2 per cent and then 4 per cent of sodium desoxycholate to yield a preparation with 5 times the activity of the original suspension.

Several lines of evidence indicate the presence of cytochrome oxidase: (1) reduced cytochrome c is readily oxidized by small amounts of the enzyme preparation; (2) cytochome c is a necessary constituent for the oxidation of hydroquinone; (3) 1×10^{-3} M sodium cyanide and sodium azide inhibit the oxidation of hydroquinone; (4) a mixture of 95 per cent carbon monoxide and 5 per cent oxygen inhibits the hydroquinone reaction by 66 per cent in the dark, and light causes an almost complete reversal of the inhibition.

Synthesis of lipids from proteins in Colpidium campylum. Charles G. Wilber and Gerald P. Seaman.

The problem of the synthesis of protein, carbohydrate and fat is a fundamental one in general physiology. Much work has been done on the problem in the vertebrates; very little on the protozoa. From the work of Mast and Pace we know that the flagellate *Chilomonas paramecium* has amazing powers of synthesis. It is also well known that lipids occur in ciliates in the form of visible globules. The types and origin of these lipids is still uncertain. Conflicting results have been obtained using the staining reactions of Nile blue sulfate. An investigation was, therefore, undertaken to ascertain the kinds and the formation of lipids in the ciliate, *Colpidium campylum.* The organisms were grown in sterile, fat-free proteose-peptone solution. Five days after inoculation, samples were taken and tested for cholesterol (*Jour. Biol. Chem.*, **24**: 227; 1916), fatty acid (*Jour. Biol. Chem.*, **77**: 53; 1928), and phospholipid (*Jour. Lab. Clin. Med.*, **16**: 158; 1930). No cholesterol was found. In a culture containing 44,000 colpidia per cc. there was 3.4 mg.% phospholipid; 2.5 mg.% was present in the cell-free culture medium. In a culture of like population there was 232 mg.% fatty acid; 183 mg.% was present in the cell-free culture medium. The results indicate that the colpidia do not synthesize cholesterol but that they produce very large amounts of fatty acid. This latter result is in accord with work done on bacteria and on rhizopods. It is suggested that fatty acid is formed in *Colpidium campylum* from the deaminated amino acid via pyruvic acid and acetaldehyde (by repeated condensations) and that glycerol is formed via pyruvic and lactic acids, glycogen, and glyceric aldehyde.

JULY 29

J. T. BONNER. No abstract submitted.

B. LIBET. No abstract submitted.

OTTO MEYERHOF AND JEAN R. WILSON. No abstract submitted.

AUGUST 5

Irreversible differentiation in certain plant cell lineages. R. BLOCH.

In ground parenchyma of the stem of *Ricinus communis* secretory cells occur the contents of which give the reactions of tannins and unsaturated fat. They appear early within the meristem of the apical growing point and, once differentiated, continue to multiply as the stem elongates. Unlike most plant tissue cells, they retain their specific physiological character during growth and division and transmit it to the daughter cells. This behavior is also evident when old internodes are wounded: the secretory cells resume growth and division, but do not dedifferentiate as other plant cells do under such conditions. They produce their own type only and exhibit stability of cell character, resembling in this, many animal cells which show similar persistence along somatic cell lineages, irrespective of external conditions.

One possible explanation of such stability of the differentiated state has been the assumption of determinants in the nature of self-duplicating cytoplasmic proteins (plasmagenes). These or their precursors could in the present case appear and come into control in certain cells as a result of differential, polar cell divisions during which cytoplasm or cytoplasmic constituents become unequally distributed or segregated.

The occurrence of such cells and of such a suggested mechanism may play a significant role in the elaboration of cellular differences in plant tissue patterns. Many cellular differences have previously been shown to arise as a result of polarity, a basic protoplasmic factor. Many others are directly induced by external physical and chemical factors in the environment which act differentially on cells in different positions in the plant body. To these may be added possible qualitative, stable changes in the cytoplasmic reactivity systems of some of the cells, accounting for some types of specific behavior.

Alkaline phosphatase activity in early development of amphibians. E. J. KRUGELIS.

The physical and physiological correlation of alkaline phosphatase activity and nucleic acids indicated from several different lines of research suggested investigation of this correlation in the developing amphibian embryo whose content and distribution of ribose nucleic acid has been thoroughly studied and described by Brachet (*Embryologie Chimique*, Liège, 1945). Using the Gomori technique, the enzymatic hydrolysis of phosphate from the substrates, sodium glycerophosphate, ribose nucleic acid, and adenylic acid was investigated in developmental stages of Rana, Axolotl, and Ambystoma. In the growing oöcyte, the enzyme activity is concentrated in the germinal vesicle, while the basophilia indicating ribose nucleic acid is in the cytoplasm. During the late cleavage stages, the enzyme reaction becomes intense in the cytoplasm of the cells of the animal pole, exhibiting the same gradient as is found for ribose nucleic acid. During gastrulation there appears a cytoplasmic and nuclear gradient from ectoderm to mesoderm to endoderm, which is similar to the one for ribose nucleic acid. Thus it appears that alkaline phosphatase is independent of ribose nucleic acid in oöcyte stages, but becomes associated with it during late cleavage. This is supplementary evidence for the hypothesis of Brachet that during development the cytoplasmic basophilic granules of ribose nucleic acid become increasingly complex by becoming associated with various enzymes, one of which is alkaline phosphatase. This increasing complexity of association of various chemical substances is probably related to morphogenesis.

J. R. SHAVER. No abstract submitted.

AUGUST 12

D. E. COPELAND. No abstract submitted.

Factors influencing the expression of "position-effects." TAYLOR HINTON.

Drosophila melanogaster was used in a study of the factors causing variation in the expression of a second chromosome inversion, Inversion (2LR)40d. The presence of this inversion results in different degrees of disarrangement of the facets of the eyes, mottling of the pigment, and deposits of a black tumor-like substance on the surface of the eyes. It was found that the sex of the individual had no significant effect upon the phenotypic expression of In(2LR)40d. The effect of different temperatures was studied and it was shown that in flies raised at 15° or 28° the eyes were almost normal; while the eyes were most affected at 23°. Crosses were made which demonstrated that the age of the parents had no influence upon the expression of the inversion. A significant difference in degree of effect was found when offspring raised in a non-crowded, well yeasted bottle were compared with offspring raised in a crowded shell vial. The former had almost normal eyes while the latter showed a marked eye effect. Females carrying a Y-chromosome in addition to the normal complement of chromosomes showed little eye effect with the inversion while their normal brothers were significantly more affected. When the inbred stock of In(2LR)40d was crossed to certain laboratory stocks the offspring showed no change in the expression of the inversion, but when crossed to other laboratory stocks a striking increase in the expression was noted. This suggests that some stocks carry genetic modifiers which enhance the expression of In(2LR)40d while others do not. Experiments are in progress to attempt to localize and study the nature of these modifiers. It can be concluded that the "position-effect" can be altered in expression by temperature, starvation, extra Y-chromosomes, and by genetic modifiers.

D. WRINCH. No abstract submitted.

AUGUST 19

Induced morphological changes in Tetrahymena vorax. C. LLOYD CLAFF.

Tetrahymena vorax, a Protozoan ciliate previously known as *Glaucoma vorax*, is normally found in Nature as a small tailed form, varying in size from 50 to 75 microns, with an oral opening just large enough to engulf its food, bacteria.

Kidder, Lilly, and Claff reported (*Biol. Bull.*, **78**, Feb. 1940) the effect of putting *Tetrahymena geleii* in association with *Tetrahymena vorax* in the same culture. Within 24 hours drastic form changes took place in the *Tetrahymena vorax:* (1) the oral opening enlarged,

(2) a large preparatory vacuole continuous with the oral opening formed, (3) the animal enlarged to a size of 100 to 250 microns, (4) it became a Carnivore and the *Tetrahymena geleii* became its prey.

It was thought it would be of interest to place a cellophane membrane between *Tetrahymena vorax* and *Tetrahymena geleii* to see if the factor responsible for this change could be shown to be diffusible.

Culture flasks were prepared as follows: Erlenmeyer flasks were modified with an extra port on the side. A Pyrex tube, enlarged at the bottom was inserted with a cellophane membrane tied securely around the enlarged flange. These were sterilized with 10 cc. of Pyrex Distilled Water in the flask, and 2 cc. of water in the tube. Then they were inoculated on one side of the membrane with Sterile *Tetrahymena vorax* grown in 2 per cent Proteose Peptone broth; and Sterile *Tetrahymena geleii* on the opposite side.

Control flasks were inoculated with *Tetrahymena vorax* on one side of the membrane and Sterile Distilled Water on the other side.

Sterility tests were routinely carried out.

The flasks were assembled on a shaker device as shown—and shaken intermittently over a period of from 24 to 72 hours.

Carnivores, or in this case Cannibals were produced in large numbers in the cultures where *Tetrahymena geleii* were introduced on the opposite side of the membrane.

No Carnivores, or in some cases only one or two Carnivores were present in the cultures where Sterile Distilled Water was present on one side of the membrane. These Carnivores were small, and did not seem to have the ability to feed properly, in as much as they contained only one or two prey, whereas the induced forms would have as many as fifty prey.

To date only 58 experiments have been performed, so this is in the nature of a preliminary report. Some interesting facts for further investigation have been disclosed.

1. The addition of particulate Yeast Harris to the cultures of vorax seems to inhibit the formation of Carnivores.
2. The addition of the bacteria Aerobacter seems to favor the formation of Carnivores.
3. There is evidence that there is some factor from the *Tetrahymena geleii* culture which is diffusible through the cellophane membrane which stimulates the formation of Carnivores.

PAUL S. GALTSOFF. No abstract submitted.

Bermuda sea urchins and their eggs. ETHEL BROWNE HARVEY.

The common species of sea urchin near the Bermuda Biological Station are: (1) *Lytechinus variegatus* (*Toxopneustes*), usually brown in Bermuda, always white in Beaufort. Decorates itself with shells, sea weed, etc. It is ripe in spring and early summer. (2) *Tripneustes esculentus* (*Hipponoë*), a very large (5–6 inches diameter) species with short white spines. It is ripe in early summer and early winter. (3) *Echinometra lacunter,* an oval reddish or brownish urchin living in holes in the reefs and known as the "rock" or "reef" urchin. It is ripe in late summer and early fall. (4) *Centrechinus antillarum* (*Diadema setosum*), having very long (sometimes 10 inches) sharp spines. This is ripe in summer and early winter. (5) *Eucidaris tribuloides,* a grayish urchin with blunt club-shaped spines, known as the "slate-pencil" urchin. Another species found near the laboratory but rare is *Echinoneus cyclostomus,* a very small urchin (one inch long), somewhat oval, cream colored with bright red tube feet. There is also a sand dollar, *Mellita sexiesperforata* (now *Leodia*) found at some distance from the laboratory.

The living eggs of *Lytechinus* are beautifully clear and many of the phenomena of fertilization and cleavage can be distinctly seen. The asters show well, but the spindle fibers cannot be observed until late anaphase and telophase, when the "interzonal" fibers can be clearly seen. The eggs of *Tripneustes, Echinometra* and *Diadema* are very granular, and the granules obscure the nuclear phenomena.

In *Lytechinus,* the stratification with centrifugal force is: yolk, clear layer, mitochondria; sometimes clear layer also at the bottom; there is usually no oil cap; in *Tripneustes:* sometimes oil, yolk, mitochondria, clear layer; sometimes oil, clear layer, yolk, mitochondria; in *Echinometra:* oil, clear layer, mitochondria, yolk. *Lytechinus* and *Tripneustes* break apart into a large nucleate fraction and a small heavy fraction.

The pluteus of *Lytechinus* has a single rod in the postoral arms, with a spiny base. The pluteus of *Tripneustes* has a lattice-like skeleton in the postoral arms, united at the base and not spiny. The pluteus of the hybrid *Lytechinus* ♀ × *Tripneustes* ♂ is maternal in the form of skeleton.

Spermatogenesis in sphecoid wasps. P. W. WHITING.

In the formation of male gametes plants exhibit no meiotic phenomena, since their haploid generation has no heritage of meiosis. The half-dozen invertebrate groups characterized by haploid males, however, exhibit, to greater or less extent, remnants of spermatocyte conditions presumably characterizing their diploid ancestors. In the hymenopteran male, meiotic phenomena are elaborate and are characteristically similar in many of the superfamilies from the lower plant-feeders to the aculeates. This "orthodox" plan consists in an abortive first division, not dividing the nucleus, and an equal second division resulting in two functional sperm cells. The bees, Apoidea, deviate in that the second division is unequal cytoplasmically so that only one spermatid functions. It has now been shown that in four species of sphecoid wasps there is but one meiotic division. This produces two equal spermatids. At the end of the growth period before this division takes place, there develops close to the nucleus, a body resembling a second nucleus of small size. This body becomes very conspicuous and persists through the meiotic division, occupying a position *within* the cytoplasm at one side of the cell. These phenomena are tentatively interpreted as vestiges of the first division still further reduced than in the bees and the vespoid wasps. In their equal second division these species resemble the latter group following the "orthodox" plan.

REPORT ON THE LALOR FELLOWSHIP RESEARCH

J. BRACHET

Arbacia eggs at various stages of development have been collected in large amounts for a cytochemical and chemical study of both types of nucleic acids: some have been fixed in Zenker's and Serra's fluids for a study of nucleic acids and amino-acids distribution, using if possible quantitative microphotometric methods. Most of the material collected will be used for chemical analysis of the phosphorus, ribose, ribodesose and purines content of the fertilized eggs, the blastulae and the plutei; this analytical work is already under its way.

Eggs and embryos treated with lithium chloride and sodium thiocyanate, in order to produce morphological defects, have also been preserved with the hope of establishing correlations between these abnormalities and the nucleic acids metabolism.

Arbacia and Psammechinus eggs have been centrifuged at various speeds and preserved in fixatives for further study of the nucleic acids and proteins.

Other experiments have shown that in sea-urchins' eggs, as in adult tissues, a large proportion of the ribonucleic acid is linked to small, ultracentrifugeable particles.

Some time has also been devoted to the study of the slides made by Dr. E. Krugelis in order to demonstrate the localization of alkaline phosphatase in developing marine Invertebrates' eggs; her results have been presented at the M.B.L.'s scientific meeting.

Finally, an abundant material has been collected with the purpose of studying the localization of ribonucleic acid and proteins in regulative and mosaic eggs: these included Asterias, Chaetopterus (normal development and differentiation without cleavage), Mactra (normal and centrifuged), Cynthia (normal and centrifuged) and Fundulus eggs and embryos.

It is proposed to study in the nearest possible future the material which it has been possible to collect during the summer months.

Comparison of the binding ability of hemocyanin and serum albumin for organic ions. I. M. KLOTZ, A. H. SCHLESINGER AND F. TIETZE.

The presence of hemocyanin in a dispersed form in the plasma of invertebrates suggests that this protein may act not only as a respiratory pigment but also as a transport vehicle for small ions. A series of studies has been made, therefore, on the binding of several organic dye ions by the serum of *Limulus polyphemus*.

In buffered solutions near pH 5 hemocyanin has been found to form complexes with the organic anion, methyl orange, though with an affinity about one-tenth that observed with the same dye and serum albumin. Thus at a concentration of free anion of about 1×10^{-4} molar, approximately 0.5×10^{-5} mole and 6×10^{-5} mole, respectively, of methyl orange is bound by one gram of hemocyanin and one gram of albumin, respectively.

No significant binding of the cationic dye, chrysoidine, by *Limulus* serum was observed even at concentrations of cation of 4×10^{-4} molar. A series of experiments with serum albumin under comparable conditions indicated appreciable binding though less than that observed with methyl orange and albumin. If the same decrease in binding in going from the anion, methyl orange, to the cation, chrysoidine, holds in hemocyanin as is found with albumin, it would require more precise techniques than were available to detect the chrysoidine complex of hemocyanin. The quantitative data on the binding of chrysoidine by albumin indicate that 16 is the maximum number of binding sites available on the protein molecule and that the first chrysoidine complex has a binding energy of 5,000 calories/mole as compared to 5,960 calories/mole for the corresponding complex with methyl orange.

These experiments indicate that hemocyanin acts not only as a respiratory pigment in the blood of invertebrates but that it also serves in the distribution and conservation of organic ions among various organs and tissues, though its effectiveness in these respects is less than that of serum albumin.

The production of diabetes in the toadfish with alloxan. ARNOLD LAZAROW AND JACK BERMAN.

Alloxan was injected into 50 toadfish (*Opsanus tau*) as a 10 per cent aqueous solution. The sugar content of the blood was determined by the Folin Malmros micro method. Serial sugar determinations were carried out on blood obtained by cutting the tail fin of the fish. The normal blood sugar value of the toadfish, which depends upon the nutritional state, varied from 26 to 86 mgs. per 100 cc. (The mean of 37 determinations on 20 fish was 54 mgs. per 100 cc.)

When alloxan was injected into toadfish the effect depended upon the dose and mode of administration. Of twenty animals injected intraperitoneally with doses of alloxan ranging from 300 to 1,000 mgs. per kilogram of body weight, only two showed blood sugar values greater than 100 mgs.% 48 hours after the injection. Histological examination of the tissues showed extensive necrosis of the liver. This apparent selectivity of alloxan for the liver (following intraperitoneal injection) may be related to the hepatic portal circulation inasmuch as a large part of the alloxan injected goes directly to the liver.

By contrast, subcutaneous administration of alloxan produced a blood sugar response in a much greater per cent of the animals. Of six toadfish injected with a dose of 400 mgs./kg., three showed a 24–48 hour blood sugar value greater than 100 mgs.% Of nine animals given 600 mgs./kg., six showed a 24–48 hour blood sugar value greater than 100 mgs.%; whereas eight of ten fish injected with a dose of 800 mgs./kg. showed elevated blood sugars at 24–48 hours. The 24–48 hour blood sugar value of animals considered diabetic ranged from 100 to 328 mg.%. Although a number of the injected animals died after 24 to 72 hours, some were kept alive for 5 days and showed a persistence of their hyperglycemia.

The urine obtained from the bladder of several fish was also examined for sugar. In two fish whose blood sugars were 200 and 268 mgs./100 cc. the urine sugar concentration was 73 and 146 mgs.% respectively.

Since the islet tissue of toadfish is segregated into discrete organs apart from the digestive pancreatic tissue, direct chemical studies can be carried out on this material. Such a study is not possible in mammals where the islet tissue constitutes a very small fraction of the pancreas. Investigations on the sulfhydryl content of the islet tissue of toadfish before and after alloxan administration will be carried out.

Localization of adenosinetriphosphatase (ATP-ase) in the giant nerve fiber of the squid. B. LIBET.[1]

It is conceivable that a basic feature of nerve impulse conduction, the permeability change in the membrane, depends upon a structural change in certain membrane proteins. If the latter resemble the myosin system of muscle, they should be associated with ATP-ase activity.

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[1] This work was carried out under a Fellowship of the Lalor Foundation, and was also supported in part by a U. S. Navy contract to the University of Chicago for research in nerve metabolism.

To test this hypothesis, measurements were made of the ATP-ase activity of the axoplasm, extruded from cleaned giant axons of *Loligo pealii*, and of the remaining sheath portion. With the tissue homogenized in 0.58 M KCl, and the activity measured in 0.58 M KCl, 0.003 M $CaCl_2$, veronal buffer ($pH = 7.4$) and 0.003 M ATP, axoplasm splits phosphate from ATP at the rate of about 0.2 γP/mg. wet weight (in 30 minutes at about 26.5° C.), while the average figure for the sheath is 19, about 100 times as much.

Not only is the ATP-ase activity of the nerve fiber almost exclusively confined to the sheath portion, but the latter has an activity even greater than that of squid muscle. Contrary to the case of cholinesterase, which is also localized in the sheath (D. Nachmansohn and B. Meyerhof, *Jour. Neurophysiol.*, **5**: 348–361; 1941), ATP-ase is not more concentrated in the optic ganglion than in the nerve.

The nerve ATP-ase also resembles vertebrate muscle ATP-ase in its substrate specificity (it did not appreciably attack hexosediphosphate, muscle adenylic acid, or glycerophosphate, and these hydrolyses were not stimulated by Ca^{++} addition). Its response to addition of varying Ca^{++} concentrations was also similar. Ca^{++} removal from the tissue by citrate or oxalate strongly inhibits the residual activity. However, nerve ATP-ase responded considerably less than muscle ATP-ase to the sulfhydryl poisons, 0.001 M p-chloro-mercuric-benzoate, chloropicrin, or p-carboxy-phenyl-arsenoxide.

Work has been initiated in the direction of attempting to make more purified preparations of ATP-ase from nerve tissue, so that the physical and chemical properties of this enzyme can be more fully compared to the myosin-ATP-ase system.

An analysis of d-amino acid oxidase in invertebrate tissues.[1] CLAUDE A. VILLEE.[2]

In the course of work on the development of a chemically defined medium for Drosophila it was found (Schultz, *Anat. Rec.*, **96**: 540; 1946) that d-amino acids seemed to be toxic to the larvae whereas l-amino acids permitted normal growth. Since the two types of isomers are metabolized by different enzyme systems in mammals, one possible explanation of this fact is that Drosophila lack d-amino acid oxidase. Experiments using dl-alanine as a substrate were unable to demonstrate the presence of the enzyme in ground Drosophila larvae (Villee, unpublished data). Studies were then made to see if this was a general phenomenon among invertebrates. The enzyme was measured manometrically using the Warburg constant volume respirometer, dl-alanine as the substrate and with the tissue suspended in a 0.05 M phosphate buffer solution at pH 7 or pH 8 (Rodney and Garner, *Jour. Biol. Chem.*, **125**: 209; 1938). The tissues used were suspensions of sea urchin sperm, sea urchin eggs, teased muscle fibers from scallop adductor muscle, spider crab claw and tail muscle, lobster claw muscle, whelk foot (white) and radula (red) muscle, and homogenates of starfish digestive gland and whelk liver. None of these tissues, from animals of three different phyla, showed any d-amino acid oxidase activity and it is possible that the lack of this enzyme is a general characteristic of invertebrates.

A study of nucleoproteins from invertebrate tissues. CLAUDE A. VILLEE.[3]

Nucleoproteins have been extracted previously from vertebrate tissues, primarily calf thymus and fish sperm, and from plant tissue, wheat germ, yeast and bacteria. The present study was undertaken to see whether nucleoproteins could be extracted from invertebrate tissues and how their properties compared with those from other sources.

Nucleoproteins were extracted by two different methods, strong salt (2 M NaCl) solution or 0.5 per cent desoxycholate solution, from squid testis, spermatophore sac, sperm receptacle and ovary; sea cucumber testis and ovary; sea urchin sperm and eggs; starfish testis, ovary and liver; whelk testis and chaetopterus sperm. All of these tissues gave viscous solutions on extraction and these, on purification and precipitation in distilled water or alcohol, gave white fibers. The most viscous solutions were obtained from extracts of squid testis and spermatophore sac; these exhibited strong birefringence of flow. When examined in the Beckman spectrophotometer, these solutions showed typical nucleoprotein absorption spectra, with a maximum at 2,600 Å. The amount of nucleoprotein not extracted by strong saline and extractable by desoxycholate is negligible. Nucleoproteins from marine animals differ from vertebrate nucleoproteins in being extracted by 2 M NaCl but not by 1 M NaCl. Analyses were made for total phosphorus, desoxyribonucleic acid phosphorus, ribonucleic acid phosphorus and phosphoprotein phosphorus by the method of Schmidt and Thannhauser and for ribonucleic acid and desoxyribonucleic acid by the method of Schneider.

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[1] I am indebted to Mr. C. Lloyd Claff for the Warburg apparatus used in these experiments.

[2] Lalor Research Fellow.

[3] Lalor Research Fellow.

The nucleic acid-protein complex of all the nucleoproteins used is dissociated in both strong salt solution and in desoxycholate solution. Loligo testis nucleoprotein in 2 M NaCl was dialyzed against 2 M NaCl for a week. At the end of that time the outer saline gave positive results with the ninhydrin and chloroform gel tests for protein, so the squid testis nucleoprotein contains a low molecular weight, dialyzable protein, presumably protamine. The nucleic acid-protein complex may be separated by the chloroform-octyl alcohol procedure of Sevag. In one experiment this procedure was repeated four times with 24-hour shaking periods each time. The resulting nucleic acid was practically free of protein; the last chloroform extract contained only minute amounts of protein. The separated nucleic acid fibers and protein powder have been washed thoroughly with alcohol and ether and dried in vacuo and will be used in further chemical studies on the nucleic acid and protein moeities by means of filter paper chromatography.

PAPERS PRESENTED AT THE MEETING OF THE SOCIETY OF GENERAL PHYSIOLOGISTS

SEPTEMBER 8 AND SEPTEMBER 9

FIRST SESSION—H. F. BLUM, CHAIRMAN

Production of a wave of negativity by acetylcholine at a phase boundary (model of the nerve impulse). T. C. BARNES.

The negative phase boundary potential generated by acetylcholine at the interface between oil (representing the lipoid layer on nerve) and saline (representing tissue fluid) affords a possible explanation of the negative potential associated with activity in the nervous system (*see* Barnes and Beutner, *Science,* **104**: 569; 1946). It was formerly believed that phase boundary potentials were direct current potentials giving a permanent shift in base line. Recent experiments indicate that a concentration of electrogenic substance too low to establish a permanent potential will set up a transient wave at the oil interface. Thus 0.05 mg. acetylcholine in 0.07 cc. saline is placed at the interface between guaiacol and saline generates a 3 mv. wave of negativity lasting 0.2 second. The equilibrium concentration in the 200 cc. of saline is I in 6 million. These waves can be repeated many times by adding more increments of acetylcholine. If the acetylcholine is added in a set-up with no oil layer there is no detectable electrical effect. These signals have been recorded on a Garceau, Rahm and Grass electroencephalograph, Cambridge electrocardiograph and by a cathode ray oscilloscope and D.C. amplifier (through the kindness of Dr. H. K. Hartline).

Controls of added saline have little or no effect, KCl gives a much smaller negativity than acetylcholine but tetraethylammonium iodide is more than twice as active as acetylcholine.

The one microgram of acetylcholine per gram present in living nerve could produce a wave of negativity if released directly on the lipoid surface.

The interrelation of the chloride excreting cell (gill) and the pseudobranch of Fundulus heteroclitus. D. EUGENE COPELAND.

Evidence of a chloride excreting cell type in the gill filaments of *Fundulus heteroclitus* is presented. The cell is columnar and usually reaches from the basement membrane to the free surface of the gill. In sea water conditions there is typically present an oval vesicle at the distal end of the cell. It opens by a small pore to the exterior. Chloride tests ($AgNO_3 + HNO_3$) give a very strong response in the vesicle and less so in the body of the cell. In fresh water conditions the vesicle is always absent. Chloride tests give a response in the cytoplasm of the tip of the cell, often giving a bar-like effect. In both conditions the cell is rich in fine mitochondria.

Observations of the gland-like pseudobranch are presented. In sea water condition the cells are typically filled with heavy, rod-like mitochondria oriented parallel to the long axis of the cell and to the free (vascular) surface of the cell. The rods are grouped parallel to each other, giving the cell an appearance somewhat like striated muscle. Preliminary results show a progressive granulation of the mitochondria in the fresh water adapting animal, reaching a peak at about twenty-four to forty-eight hours, then returning to a rod-like condition almost like that found in the sea water condition. In sea water adapting animals there is progressive rod-like organization, reaching the typical sea water picture in about twenty-four hours. Three lines of evidence suggest a functional relationship of the pseudobranchial and chloride cells. (1) Homology. Both cells are acidophilic. Leiner (1938) has demonstrated vesicles in the pseudobranchial cells of *Smaris alcedo.* (2) Leiner (1938) has localized carbonic anhydrase in pseudobranch. This might aid in the Cl shift in the chloride cell. (3) Differential behavior of mitochondria described above may show a participation in adaptation.

Some physiological factors involved in the maximum velocity at which trout swim. Kenneth C. Fisher.

It is well known that trout tend to orientate against a current of water and that they will swim actively so as to maintain themselves in a constant position. If the current is gradually increased, a point is reached above which the fish cannot swim fast enough to maintain the constant position. The physiological factors which determine this limiting velocity are under investigation.

Rogers showed, some years ago in this department, that the velocity varies with temperature and, in fact, that it is a maximum at about 10–15° C., far below lethal temperatures. We find now that the frequency of "spontaneous" movements made by fish in still water also varies with temperature and shows a maximum at 10–15° C. Furthermore, the limiting velocity for fish in a current may be varied in a simplified environment by changing the light intensity. It is, therefore, tentatively concluded, that the limiting velocity is determined, in the first instance, by the frequency of swimming movements; that the latter occur due to "spontaneous" or other stimuli and may then vary in frequency or in strength in accord with the strength and frequency of these stimuli; and finally, that the response to the stimuli received is modified in accord with the ambient temperature by the activity of the body surface temperature receptors.

Protoplasmic injury substances and their effect on the organism. L. V. Heilbrunn and Louis Moreau (*by invitation*).

Earlier work from this laboratory has shown that when tissues of various organisms are injured, substances are produced which have a thrombin-like action in causing the clotting of protoplasm in the absence of calcium. We believe that the production of thrombin-like or thromboplastic substances by injured tissues may help to explain some of the baffling problems of clinical physiology. In earlier experiments we used the protoplasm of sea-urchin eggs to test for the presence of thrombin-like substances. In recent work we have used blood clotting times as an indicator. For this purpose the frog is a useful experimental animal. When frog tissues are injured, the frogs show typical shock reactions, but the problem of studying these shock phenomena is comparatively uncomplicated, for stoppage of respiratory movements or even stoppage of circulation has little immediate effect. We have developed methods for studying the clotting time of frog blood. When frogs are subjected to violent head injury, the blood, when examined immediately, shows shortened clotting time, indicating the release to the blood of thromboplastic substance. Some minutes later, there is a counter reaction and the clotting time of the blood is prolonged. Injury due to electric currents can also produce changes in the blood clotting time. Injury substances may be released from the brain or from the muscles. If the muscles of the legs are injured by electric currents, the effect on the frog as a whole may be inhibited by ligaturing the upper thighs. We have begun to work also on heat injury. In general, the results indicate strongly that various types of tissue injury release thrombic or thromboplastic substances to the blood stream, and that these substances can affect tissues throughout the organism.

Preparation and properties of cation-permeable erythrocytes. M. H. Jacobs and Marian Willis.

It has long been recognized that unrestricted permeability of an erythrocyte to both anions and cations would be osmotically incompatible with its continued existence. The view has more recently been expressed by several workers, particularly by Wilbrandt, that certain so-called "non-osmotic" hemolytic agents may act through the creation of a condition of cation permeability, resulting in an osmotic unbalance between the erythrocyte and its surroundings. The present paper is concerned with the mode of action of one such hemolytic agent, n-butyl alcohol, which shows in a particularly clear and uncomplicated manner some of the consequences of an induced cation permeability.

Erythrocytes having different chosen degrees of porosity, as measured by a series of non-electrolytes of increasing molecular volume, may be prepared by exposure for different lengths of time to a solution of 6 volumes per cent of butyl alcohol in isotonic NaCl, sucrose, or both, followed by dilution with and, if desired, by subsequent washing in a pure sucrose or sucrose-NaCl solution. The cells so prepared obey very satisfactorily predictions made from newly derived equations for the theoretical ionic and osmotic equilibria between cation-impermeable and cation-permeable cells and solutions containing NaCl alone, sucrose alone, and both substances together.

Among the theoretical properties of cation-permeable cells shown by erythrocytes treated with butyl alcohol are the following: (1) unlimited swelling in all concentrations of NaCl, (2) strong and rapid shrinkage in pure isosmotic sucrose solutions, (3) assumption of definite intermediate volumes in most mixtures of NaCl and sucrose, with protection against hemolysis by extremely low concentrations of sucrose, (4) more rapid swelling rather than shrinkage on alkalinization of an external NaCl solution, and (5) shrinkage followed by swelling beyond the original volume on the addition of concentrated NaCl to an appropriate external mixture of NaCl and sucrose.

Evidence of active transfer across the human erythrocyte membrane. PAUL G. LEFEVRE.

Following an earlier report concerning the effectiveness of various sulfhydral inhibitors in delaying hemolysis of human red cells in isotonic glycerol, the course of the volume changes upon addition of glycerol to suspensions of these cells in isotonic salt or sugar solutions was followed in the Parpart apparatus. The inhibitory action of cupric and mercuric ions, iodine, and p-chloromercuribenzoate upon the rate of entry of glycerol, and the reversibility of this inhibition by sulfhydryl groups, was thus verified. Similar inhibitory effects upon the exit of glycerol from cells previously equilibrated with glycerol solutions was observed. The glucoside phlorizin, usually considered inhibitory to phosphorylations, exhibited a similar effect; chemical methods, however, indicated that no inorganic phosphate was consumed in the accumulation of glycerol into these cells.

The slow swelling of erythrocytes in solutions containing glucose was also delayed by phlorizin, and was particularly sensitive to mercuric ion and to p-chloromercurimenzoate, although cupric ion showed no inhibitory effect whatever. Comparison of the volume changes upon addition of various increments of glucose to suspensions of cells in isotonic saline showed that the rate of swelling was inversely proportional to the sugar concentration at the higher concentrations, but followed the usual exponential form with the lower concentrations; this set of relations indicated that there was a limiting rate at which glucose could enter these cells, regardless of the magnitude of the concentration gradient. Previous equilibration of the cells with glucose at 0.05–0.1 M greatly delayed the subsequent entrance of glucose when the external concentration was raised; this suggests that the postulated transfer mechanism functions only when the intracellular concentration is below a certain critical concentration in this range. These results have been partly duplicated with galactose or levulose in place of glucose, and have been verified by chemical analyses of the glucose content of the suspending medium.

Phosphatase activity in the chloride cells in the gill of Fundulus heteroclitus. OLIVE PETTENGILL.

A histochemical examination of the chloride cells of the gills of *Fundulus heteroclitus* which had been adapted to both fresh water and salt water was made, using the Gomori technique. Material was fixed in cold 80 per cent alcohol or acetone, dehydrated and embedded in the usual manner. The substances used which gave a positive reaction were yeast adenylic acid, yeast nucleic acid, hexose diphosphate and the standard, beta-glycerophosphate.

There is a strong activity in the fresh water gill, localized generally at the distal end, between the nucleus and the end of the cell, and the cell seems to be enlarged. In sea water, the vesicle, which is characteristic of this condition, is surrounded by a very dark ring of activity, the signet ring effect. The total amount of activity is less than that seen in fresh water.

Copeland (1947) has presented evidence to support the theory that this cell does excrete chloride in the salt water fish. One might conclude that the dark ring of phosphatase activity around the chloride-excreting vesicle may indicate an increase in metabolic activity necessary to transport the chloride from the cell into the vesicle. This would constitute a physiological mechanism to remove chloride from the body.

Krogh (1939) suggests that euryhaline fish such as Fundulus may have a double mechanism in the gills that would pump chloride out of the body under sea water conditions and into the body under fresh water conditions. It is possible that the two mechanisms are represented in a single cell. The strong phosphatase activity in the fresh water form may indicate a reversal of the functional polarity of the cell with consequent pumping of chloride into the body. The increased amount of phosphatase activity may be indicative of the greater osmotic gradient encountered in the fresh water condition.

SECOND SESSION—A. K. PARPART, CHAIRMAN

Permeability to radioactive sodium in frog eggs. WILLIAM R. DURYEE AND PHILIIP H. ABELSON.

It is the main purpose of this paper to show how much and how fast radioactive sodium (Na^{24}) exchanges with normal sodium (Na^{23}) in a typical vertebrate egg. Secondary purposes are to show where the sodium goes inside the cell and to throw some light on the regulative mechanism. Permeability has heretofore been considered chiefly a membrane phenomenon. Our results indicate that permeability to sodium is really a more complex matter in that diffusion through cytoplasm and blocking of internal exchange are the main limiting factors. Due to high activity of the Na^{24} plus sensitive counters results can be expressed accurately to $\pm .1 \times 10^{-8}$ grams Na.

Ovarian eggs of *Rana pipiens* were cut apart and exposed to various Ringer solutions made up with Na^{24}. Control solutions were made with identical amounts of normal sodium. The Na^{24} was made by bombardment in the cyclotron. Our criteria of actual penetration were three-fold: (a) by radio-autographs of sections of eggs that had been frozen in liquid air, (b) by puncturing the cell to remove aliquots of the yolk-cytoplasmic mass for direct measurements with a Geiger counter, and (c) by isolation of the cell nucleus for direct measurement.

A marked difference was found between the up-take curves for live and dead cells. With the former radio-sodium rises rapidly in concentration to a value of 2.5×10^{-7} gm. Na in 30 minutes and tends to level off. At the end of 4 hours the average concentration is 3.7×10^{-7} gm. Na per egg. In the case of dead cells Na^{24} diffuses in more rapidly reaching a concentration of 38×10^{-7} gm. in 30 minutes and then levels off. These "terminal values" correspond roughly with the total sodium per egg found by direct chemical analysis of the ovaries. It is concluded that all the sodium in a dead egg is exchangeable, the membrane forms practically no barrier and Na once in the egg diffuses freely. In a live egg only 10 per cent of the total sodium is exchangeable.

From washing experiments with live eggs it is clear that there are two kinds of sodium in the cell—one freely mobile and the other slowly exchangeable over a period of ten or more hours. Calcium-free or double calcium Ringer solution did not affect the initial rate or quantity of exchange of Na^{24}, but after several hours a deficiency in calcium permits an exchange of sodium to a level of 6×10^{-7} gm. as compared with a level of 3×10^{-7} gm. Na per egg immersed in a Ringer with twice the amount of calcium.

From radio-autographs it is evidence that distribution of sodium is relatively uniform throughout the cytoplasm in less than 30 minutes, but that it is strikingly concentrated inside the nucleus. This shows that the nuclear membrane is readily permeable to Na^{24} and that the amount of sodium available for exchange is approximately twice as great in the nucleus as in the cytoplasm.

Accumulation and distribution of radioactive materials in goldfish. C. LADD PROSSER.

In experiments at the Metallurgical Laboratory, University of Chicago, the uptake of products of uranium fission and of radioactive sodium by goldfish was measured. Accumulation of radioactive strontium, barium, rare earths and sodium was rapid initially (during the first few hours of immersion) and continued at a rate greater than the loss of radioactivity in the medium by decay and adsorption. The ratio of the concentration of active material in the fish to that in the solution increased with time. This concentration ratio was usually between 10 and 100. The concentration ratio was independent of dose over the range investigated (0.01μ C to 1.0μC/ml.).

Calcareous tissues, such as skeleton and scales, accumulated about 75 per cent of the radio-strontium and radio-barium in the whole fish. Rare earths were accumulated in the visceral tissues, especially the intestine, and were more strongly adsorbed on feces and glass than the alkaline earths. The comparable distribution, particularly of bone-seekers, on immersion and injection, indicated that these elements were taken up through gill and oral membranes and were distributed by the blood. Radio-sodium was present in greater proportion in the gills at six hours than later. Some fission elements, particularly the rare earths, appeared to be precipitated along the intestinal folds. The yolk of eggs in ripe ovaries accumulated rare earths but not strontium or barium.

Young growing or regenerating bone accumulated more radioactive material than did older bone; cartilage accumulated none of the bone-seekers. Uptake of Sr^{89} was greater when the surrounding pond water was low in calcium (1.8 p.p.m.) than when it was high in calcium (36 p.p.m.). Stable strontium also retarded the uptake of radio-strontium but to a less degree than calcium.

When the fish were transferred from active to inactive pond water they failed to lose their radioactivity faster than by decay. The rate of loss of radio-strontium appeared to be unaffected by the calcium content of the water. Goldfish were unable to remove appreciable quantities of fission elements from suspended clay.

Third Session—J. H. Bodine, Chairman

A method for subcooling cells under microscopic observation at room temperature. Robert Chambers and Silvio Beaz (*by invitation*).

The principle of the technique is to cool the objective of the microscope so that the objective serves as the refrigerator to sub-cool an oil-enclosed hanging drop, containing the specimen, suspended from a coverslip which roofs the micromanipulation chamber on the stage of the microscope.

The cooling is accomplished by means of a closed, circular, metal trough placed around the objective, and communicating with a tank of compressed gas. The inner wall of the trough is perforated with holes to permit the expanding gas to blow on the objective.

The chamber on the stage of the microscope is hermetically sealed off and the movements of a microneedle in the chamber are made possible by having it pass through a mercury-filled five to ten mm. hole in a brass plate which walls off the end of the chamber directed toward the micromanipulator.

An essential feature is to prevent fogging of the optical system of the microscope. This is accomplished by having drierite ($CaCl_2$) in a trough inserted into the lower end of a wide-barrelled drawtube of the microscope. The front lens of the objective is kept dry by the escaping gas rushing about it. The space within the closed chamber is also dried to minimize the formation of water condensation on the hanging oil drops.

The temperature is regulated by varying the rush of the gas and by operating an electric microheater consisting of a platinum or nichrome strip of wire in the chamber joining the ends of two copper wires connected with a dry battery cell.

The temperature is determined by the congealing of one or more of several drops of oil-mixtures suspended in the chamber. The temperature at which the congealing occurs has been previously determined by preparing paraffin wax (50° C. melting point) dissolved in Nujol oil at different percentages.

The hanging drops enclosed in oil cool below their freezing points and freezing is accomplished by "seeding" the drops or the enclosed cells by means of the tip of a microneedle.

Preliminary experiments on cells indicate that the icicles which form make a pattern in accordance with the structure in a living cell.

The possible role of peroxidase in cellular respiration. DAVID R. GODDARD AND ANNE M. SLATER.

The respiration of horse radish root sections is inhibited by low concentrations of cyanide, azide, and hydroxylamine; and photoreversibly by carbon monoxide. The inhibition results are identical with those obtained with cytochrome oxidase plants, but extracts fail to show any catalytic activity with cytochrome c and hydroquinone, and cytochrome oxidase is presumably absent. The experiments appear to establish the respiratory role of peroxidase in this tissue, in which the peroxidase is functional with molecular oxygen, that is, it functions as an oxidase. The carrier is unknown, but is not dioxymaleic acid, as this compound brings about an extra oxygen consumption of one atom per molecule of added substrate. The dioxymaleic acid oxidase is probably an artifact.

Resistance of cell membranes to internal pressures. PETER RIESER (*introduced by D. V. Heilbrunn*). READ BY TITLE.

In connection with other studies from this laboratory on the magnitude of the osmotic forces to which a cell membrane is exposed, an attempt has been made to determine in general how much pressure the membranes of cells can withstand without rupturing. Accordingly, cells were microinjected and the pressure created by the injection was determined. In order to accomplish this, a dial-type pressure gauge was connected to the injection circuit in close proximity to the micro-pipette. Eggs of the sea-urchin *Arbacia punctulata,* the starfish *Asterias vulgaris* and the annelid worm *Chaetopterus pergamentaceus,* as well as isolated muscle fibers of the frog *Rana pipiens,* were used in the experiments. The plunger of the microinjection syringe was pushed slowly either by hand or with the aid of a screw device, and the injection fluid—sea-water for the marine eggs and Ringer's fluid for the muscle fibers—was forced out of the pipette. In order to provide for ready visibility, the injection fluids were colored with methylene blue. When the pressure became too great for the cell membrane to withstand, the membrane ruptured. Typically the break was immediately sealed by the surface precipitation reaction. There was rarely any large volume increase of the cell. When the cells ruptured, the pressure was read off on the gauge. The values on the gauge ranged from 40–175 mm. Hg. Actually, these pressures recorded on the gauge are higher than the pressures acting within the cell, for the flow of fluid through the pipette would tend to reduce the pressure existing at the tip of the pipette. Thus one may conclude that for various types of cells the pressure necessary to rupture the cell membrane is typically less than 40–175 mm. Hg. It is hoped later developments will give more exact rather than maximum values.

The effect of temperature change on the oxygen consumption of yeast. F. G. SHERMAN.

The effect of changing the temperature on the rate of oxygen consumption of a bottom yeast (American Type Culture Collection No. 9080) was determined with a Warburg respirometer. The yeast was grown aerobically at 30.1° C. for 24 hours in a modification of the medium described by Nickerson and Carrol (1943). After harvesting, the yeast was washed three times with M/15 KH_2PO_4 and resuspended in this buffer. Glucose was tipped in at zero time from the side arm of the vessels. Controls were run at 30.1° C. Experimental vessels were placed in the 30.1° bath for two hours and then removed to the cold bath for 24 hours after which they were returned to the warm bath. A thermobarometer was transferred with the vessels when they were removed from one bath to the other. Three series of experiments were run with the warm bath at 30.1° C. and the cold bath at 5.2° C., 10.6° C. and 14.8° C., respectively. The control and experimental vessels were run in quadruplicate in each experiment.

In every case the yeast which had received cold treatment began to consume oxygen at the same rate as the controls when they were returned to the warm bath. In no case was an overshoot or undershoot observed in this yeast under the conditions of these experiments.

Studies of the metabolism of normal and mutant imaginal discs of Drosophila. CLAUDE A. VILLEE.

A basic problem of physiological genetics is how do embryonic tissues which are going to develop into different organs differ in their enzyme constitutions. The imaginal discs of Drosophila, discrete groups of cells developing into particular adult structures, provide a means of attacking this problem. One approach in analyzing the effects of a gene in development is to determine its effect on metabolic activity as reflected by the rate of oxygen consumption. This has been done by means of the Cartesian diver ultramicrorespirometer. Previous work (*Proc. Nat. Acad. Sci.*, **32**: 241; 1946) showed that normal wing discs have a Q_{O_2} of 20; "miniature" wing discs, a Q_{O_2} of 18; and "vestigial" wing discs, a Q_{O_2} of 9. The leg discs of all three stocks have a Q_{O_2} of 20. On the hypothesis that each gene regulates a particular biosynthesis, a search for the enzyme affected by the "vestigial" gene has been made by adding specific inhibitors or substrates to the buffer solution containing the disc in the diver. Normal and "vestigial" disc respiration is inhibited by cyanide and azide and thus is mediated by an iron or copper porphyrin system, probably the cytochromes. Respiration is also inhibited by the hydroxynaphthoquinones SN 5949 and SN 5090, believed to inactivate some enzyme between cytochromes b and c. The respiration of "vestigial" discs is increased to that of normal discs by ascorbic acid and to a lesser extent by p-phenylene diamine and hydroquinone. These experiments indicate that the respiratory enzymes of the developing imaginal discs of insects are similar to those of a wide variety of cells from bacteria to mammals, that the cytochrome oxidase system of "vestigial" is normal and that the "vestigial" gene affects some enzyme below cytochrome c in the respiratory chain.

The oxygen consumption of the eye discs of wild type, Bar, and Double Bar larvae have been measured similarly; at two hours before pupation their uptakes are 5.7, 7.3, and 8.2 mlμ. O_2/hr./disc, respectively. The Bar and Double Bar discs are slightly smaller than wild type, so the differences on a Q_{O_2} basis would be even more striking. Thus, in contrast to m and vg wings, where the structures with smaller adult sizes have a lower Q_{O_2} in the imaginal disc, the B and BB eye discs have a higher rate of oxygen consumption than the normal eye discs although the resulting eyes are smaller than normal. The addition of 0.02 M succinate to the substrate increases the oxygen consumption of all three types but the greatest increase occurs in wild type: 14.2, 10.2, and 8.8 mμl. O_2/hr./disc for wild, B and BB, respectively. The eye disc respiration is also inhibited by cyanide, azide and the hydroxynaphthoquinones.

Lipid metabolism in the protozoa. CHARLES G. WILBER AND GERALD R. SEAMAN.

It is well known that protozoa contain fat in the cytoplasm and there is some evidence that fat can be synthesized by these organisms. Identification of fat has previously depended on staining reactions with Nile blue sulfate or with Sudan dyes. In order to study the synthesis of fat in ciliates, sterile cultures of *Colpidium campylum* were grown on fat-free proteose-peptone for various periods of time. Lipids were estimated by standard biochemical methods: fatty acid by oxidation, phospholipid and cholesterol colorimetrically. The population of the cultures reached a maximum of about 65,000 colpidia/cc., 48 hours after inoculation; it remained constant until the fourth day and then rapidly decreased. At all times the amount of fatty acid and of phospholipid remained constant in the organisms themselves. Fatty acid in the cell-free culture fluid increased linearly until the 4th day, remained constant until the 6th day, and finally increased to a maximum of 313 mg./100 cc. on the 8th day. Phospholipid in the culture fluid increased linearly until the 3rd day; from the 3rd to the 4th day there was a tremendous increase in phospholipid from 1.6 mg. lipid P/100 cc. to 3.8 mg. lipid P/100 cc. The phospholipid then remained constant until about the 6th day after which it increased to 5.3 mg. lipid P/100 cc. on the 8th day. At no time was cholesterol found in the organisms or in the culture fluid. The results indicate that colpidia can synthesize lipids from purely protein sources. There is also evidence the sudden increase in phospholipid foreshadows the decrease in population.

It is suggested, as a working hypothesis, that the so-called "growth substance" reported by various workers is lipid in nature. Whether cholesterol, which has been reported to be carcinogenic in action, influences the growth in *Colpidium* is not at present known. Experiments are in progress to ascertain the effect of cholesterol and of other lipids on metabolism and growth in *Colpidium campylum*.

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THE RESPIRATORY QUOTIENT OF DROSOPHILA IN FLIGHT

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INTRODUCTION

In view of the unusual performance of insect flight muscles, which are able at times to make over a million successive contractions at rates up to several hundred per second, information on the biochemical transformations which supply the energy for this intense activity is of special interest. The purpose of this study has been to ascertain what evidence as to the types of substrate utilized during flight could be obtained from continuous measurements of the respiratory quotient.

The earlier students of the respiration of flying insects, whose results have been reviewed in detail by Jongbloed and Wiersma (1934), were generally content to assume a respiratory quotient of 1. Jongbloed and Wiersma were the first to provide an experimental basis for this theory when they allowed individual bees to fly for periods of about 5 minutes in closed containers and showed that oxygen was consumed and carbon dioxide given off in nearly equal amounts. Beutler (1936 a, b; 1937) then demonstrated that bees and some other Hymenoptera were dependent upon sugar for the ability to fly, and in fact for the maintenance of life, but because of the specialized physiology of bees and their adaptation to the food stores of the hive one is hesitant to transfer these findings without further support to other types of insects.

Nevertheless there is evidence that in the Diptera also the flight respiratory quotient is 1. Thus, Chadwick and Gilmour (1940) in two short flights of 5 and 6 minutes duration found with *Drosophila repleta* an equivalence of oxygen uptake and carbon dioxide output, while Williams and colleagues (1943) were able to show for *D. funebris* and *Lucilia sericata* a quantitative correspondence over a period of 60 to 90 minutes between the rate of disappearance of glycogen and the amount of flight activity as measured by the rate of wing-beat and the duration of flight. Whether carbohydrate was the only fuel consumed during these longer flights was not determined. Studies of flight respiration by Davis and Fraenkel (1940) and by Krogh and Zeuthen (1941), the most recent known to the writer, were concerned primarily with other aspects of the problem, and do not afford a basis for answering this question. Therefore, since the technique of Fenn (1928) provides a means of making continuous simultaneous measurements of oxygen consumption and carbon dioxide production, it seemed worthwhile to combine this method with stroboscopic determinations of the rate of wing-beat in order to study possible fluctuations in the respiratory quotient as related to activity, particularly in the later stages of prolonged flights.

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* These experiments were done while the writer was a member of the Department of Physiology of the University of Rochester School of Medicine and Dentistry.

MATERIAL AND METHOD

The procedure followed differed little from that described by Chadwick and Gilmour, except that the Warburg manometers of their experiments were replaced by a differential volumeter with side tube for conductivity measurements, of the type devised by Fenn. The volume of each vessel was approximately 30 ml.; the capillary was 30 cm. long and had a capacity of about 5 cu.mm. per cm. CO_2 given off by the insect was absorbed in 3 ml. of 0.01–0.02 M $Ba(OH)_2$, which covered the bottom of the experimental vessel and was tipped into the side tube for measurement of the change in impedance at intervals of 1 minute while flight was in progress. Simultaneous records of O_2-consumption were obtained from the movement of an index drop in the capillary which connected the control and experimental vessels, while wing-beat frequency was measured stroboscopically, as described previously, at 10-second intervals.

The sensitivity of the measurements is estimated as follows:

CO_2 : ± 0.02 cu.mm. (= 1 scale division on slide wire of conductivity bridge)
O_2 : ± 0.025 cu.mm. (= 0.1 mm. on capillary scale read with aid of a magnifying glass)
wing-rate : ± 1 per cent (manufacturer's specification for stroboscope)

Relatively greater errors were introduced by imperfect synchrony between the three types of measurement, since, for example, a lag of 30 seconds between recordings of oxygen and carbon dioxide would place the second observation in excess by 50 per cent of the respiratory rate during the last minute of the period of measurement. The observers gave special attention to eliminating this source of error in so far as possible, so that when the measured amounts of oxygen or carbon dioxide have been summed for periods of 5 or more minutes, the uncertainty from this cause may be estimated conservatively at not more than 2 per cent of the totals.

It is more difficult to take account of lag in the absorption of carbon dioxide under the conditions of the experiments, where an insect of not more than 1.5 mg. weight in a vessel of 30 ml. capacity was using oxygen and producing carbon dioxide at variable rates up to nearly 1 cu.mm per minute. Absorption curves were determined for the vessel and showed that when a few cu.mm. of CO_2 were introduced 93–95 per cent was absorbed in 5 minutes, but these could give only an approximation to the actual experiments, the purpose of which was to follow continuously the unknown and unpredictably changing rate of gaseous exchange of the animal. Since the rate of absorption will be proportional at any instant to the concentration of gas existing in the vessel, identical quantities of carbon dioxide liberated over a given interval of time will give different percentages of absorption by the end of the period, if the lag in absorption is appreciable, depending on whether the rate of liberation was constant, rising or falling. Thus one would need independent knowledge of the rate of change in rate of CO_2-production in order to apply satisfactorily any absorption "constants" which had been determined for the system. For this reason, no attempt has been made to correct the data presented below.

Lag in absorption of carbon dioxide not only introduces an asynchrony between the record of respiratory exchange and the measurements of wing-beat frequency when sudden changes occur, but may result also in spurious values for the respiratory quotient. Consider, for instance, the consumption of 100 units of oxygen and the production of 90 units of carbon dioxide during an interval in which only 70 per cent of the carbon dioxide produced is absorbed. Readings taken at the beginning and end of this period would indicate a carbon dioxide production of only 63 units; the remaining 27 units of this gas are still in the vessel, however, and occupy space vacated by a similar number of units of oxygen. Consequently the volumeter records the disappearance of only 73 units of oxygen. The apparent respiratory quotient for the period will therefore be given by the ratio 63:73 (= 0.86), whereas the true value is 0.90. Thus, the apparent respiratory quotient is depressed below the true value, if this is less than 1, and elevated above it, if it is really greater than 1. In many experiments lag in absorption will not lead to much error in the estimation of the respiratory quotient, since, if the rate of production of CO_2 remains constant, the concentration of the gas in the vessel and consequently the rate of absorption will rise, so that in course of time the apparent respiratory quotient will approach the true value. But in experiments with insect flight, it is often impossible to allow time for equilibration after changes in the rate of production of CO_2

Evidently, if there had been no other factors to be considered, it would have been preferable in this study to have used vessels small enough to make the lag in absorption of CO_2 insignificant. But to have done so would have required rather drastic changes, which have not yet proved feasible, in the technique of the conductivity measurements. The data obtained with the larger vessel are therefore presented here and are to be interpreted with appropriate reservations, especially for periods in which marked changes occurred in the respiratory rate, such as at the beginning and end of flight.

As specimens for the study, adult males of known age were taken from cultures of *D. virilis* and *D. americana*, which were kindly supplied by Dr. H. D. Stalker. The animals were reared in the laboratory (temperature 20°–25° C.) in half-pint bottles on a standard medium. In preparation, the flies were immobilized with ether and the dorsal tip of the abdomen fastened with paraffin to a paper mount which was later affixed to the stopper of the respirometer vessel. Only one specimen was run at a time. The animal rested in the head-down position in the vessel, with the feet in contact with a light spring platform which could be retracted by means of an electromagnet situated outside the water bath in which the respirometer was immersed. Control measurements were made in order to ensure that the respiration recorded was that of the animals rather than the apparatus.

All experiments were run at a bath temperature of 20.0 ± 0.01° C. After a half-hour for equilibration, the resting respiratory exchange was followed for a considerable length of time (see Table I) before the animal was stimulated to fly by withdrawing the platform on which his feet were supported. Flight continued until it ceased spontaneously, after which the resting exchange was again measured for a number of hours during the post-flight period. The animal was weighed at the conclusion of the experiment.

These experiments would not have been possible without the interest and cooperation of Professor Wallace O. Fenn, in whose laboratory they were carried out. I am also indebted to my wife for assistance with the measurements.

TABLE I

Preflight respiratory exchange in Drosophila

Specimen number	Weight	Age	Duration of measurements	Average CO_2-output	Average O_2-uptake	Average R.Q.
	mg.	days	min.	cu.mm./gm./min.		
1	1.5	0–2	120	39.7	26.8	1.48
2	0.9	2	188	31.5	25.1	1.25
3	0.9	2	71.5	48.5	32.2	1.51
4	1.0	2	148	40.0	29.2	1.37
5	1.0	5	1460	20.6	27.6	0.75
6	0.7	2	253	35.6	32.1	1.11
7	1.1	2	102	33.4	28.7	1.16
8	0.9	2	181	30.8	19.9	1.55
9	1.5	2	380	23.2	18.8	1.23
10	1.2	3	369	28.6	23.1	1.24
11	1.4	2	294	28.1	25.0	1.13
12	1.1	5	367	25.3	18.2	1.39
13	1.5	7	369	20.8	22.1	0.94
14	1.2	2	191	37.2	35.2	1.06
Average				31.7	26.0	1.23

Specimens numbered 12, 13 and 14 were males of *D. virilis;* the others, males of *D. americana.*

RESULTS

a. Respiration before flight

Data on the respiratory exchange during the period before flight are given in Table I, in which the age and weight of the specimens are also recorded.

The average rate of oxygen consumption (26.0 cu.mm. per gm. per min.) is in good agreement with resting values which had been determined for *Drosophila* by other methods (Kucera, 1934; Chadwick and Gilmour, 1940). Comparable figures for production of CO_2 seem not to have been published previously.

b. Respiration during flight

Of the 14 flies listed in Table I, 6 flew continuously for periods of 56 to 154 minutes. The respiratory data obtained have been summed for each of these individuals over successive intervals of approximately 20 minutes, and are shown together with the corresponding average rates of wing-beat and the calculated respiratory quotients in Table II. The correlation between rates of wing beat and of respiration is illustrated graphically for 4 of these animals in Figure 1, where each point plotted represents approximately 5 minutes of flight. The average rate of oxygen consumption during these long flights was about 14 times, and of CO_2 production about 11 times the previous resting rate; in the earlier study with *D. repleta* the average oxygen consumption during flight was 13 times the value at rest.

Table II

Respiratory exchange of Drosophila in successive periods of longer flights

Specimen number	Duration of measurements min.	Average frequency beats/min.	Total CO_2-output cu. mm.	Total O_2-uptake cu. mm.	Average R.Q.
1	22	9208	17.14	16.09	1.06
	22	9125	16.39	17.68	0.93
	21	8669	13.75	14.34	0.96
	22	6927	8.82	9.31	0.95
3	21.5	9233	8.21	7.17	1.14
	20	7892	5.81	5.82	1.00
	20	7580	5.82	5.61	1.04
	9.3	7004	1.95	2.32	0.84
10	20.3	9345	14.48	13.89	1.04
	20	8721	11.67	11.36	1.03
	22	7860	10.68	9.98	1.07
	21.3	7827	10.13	10.45	0.97
	21	7729	9.29	9.77	0.95
	20.5	8065	10.88	11.03	0.99
	20.2	8181	11.10	11.69	0.95
	8.8	8201	4.97	4.66	1.07
11	19.0	9111	11.60	11.92	0.97
	22.3	8219	12.60	12.41	1.01
	18.7	7071	7.10	7.79	0.90
	22.2	6756	7.95	8.09	0.98
	20.2	6519	7.41	7.56	0.98
	22.7	6638	7.14	8.06	0.89
	21.5	6354	6.19	6.30	0.98
13	20.2	7659	8.83	8.96	0.98
	17.5	7450	8.09	7.30	1.11
	18.0	6917	6.48	6.09	1.06
14	21.0	8289	9.90	10.29	0.96
	17.3	7128	8.59	8.69	0.99
	13.2	7869	6.52	6.78	0.96
	12.8	7632	5.87	5.94	0.99

Performance in shorter flights is depicted adequately by the data for the initial periods of those flies which flew for longer times, and was covered also in the earlier publication.

c. Respiration after flight

Irregularly spaced measurements of oxygen consumption and carbon dioxide production after flight were made on the 6 individuals which flew for long periods. These have been recorded in Table III. Differences between the rates determined after flight and the average rates before flight are shown in columns 5 and 6 of the table.

TABLE III

Respiratory exchange of Drosophila after long flights

Specimen number	Interval of measurement	Rate after flight		Excess over rate before flight		Postflight R.Q.
		CO_2	O_2	CO_2	O_2	
	min.	cu.mm./gm./min.		cu.mm./gm./min.		
1	0–5	75	128	35	101	0.58
	5–10	39	36	−1	9	1.07
	10–30	25	41	−15	14	0.60
	30–50	23	31	−17	4	0.76
	50–92	22	45	−18	18	0.47
	92–1065	17	31	−23	4	0.56
	1065–1133	17	38	−23	11	0.46
	1133–1199	15	21	−25	−6	0.72*
	1199–1231	16	16	−24	−11	1.00*
3	0–4.7	161	117	112	85	1.39
	4.7–25.7	41	77	−8	45	0.54
	25.7–52.7	17	31	−32	−1	0.57
10	0–5	145	140	116	117	1.04
	5–14	53	89	24	66	0.60
	14–20	42	58	13	35	0.72
	20–31	34	36	5	13	0.96
	31–787	19	28	−10	5	0.66
11	0–13	66	83	38	60	0.79
	13–23	33	29	5	4	1.12
	23–43	25	24	−3	−1	1.04
	43–59	26	25	−2	0	1.04
	59–70	23	30	−5	5	0.76
13	0–8.5	110	85	89	63	1.28
	8.5–20.5	53	43	32	21	1.22
	20.5–37.5	32	29	11	7	1.11
14	0–8.3	92	87	55	52	1.06
	8.3–22.7	44	42	7	7	1.06
	22.7–50.2	32	39	−5	4	0.83
	50.2–75.2	34	38	−3	3	0.90

* Specimen moribund.

Rates are given to the nearest whole number; R.Q. computed from actual observations.

DISCUSSION

a. Respiration before flight

The most striking feature of the preflight respiration was the high respiratory quotient, which averaged less than 1 with only 2 of the 14 individuals examined (Table I). So far as could be determined, these observations were not the result of any artefact in the experiments and at first it was thought possible that the flies might be giving off ammonia as an end product of protein breakdown. If this should occur, the volumetric determinations of oxygen consumed would yield erroneously low values, while there would be no interference with the absorption of CO_2. The possibility was ruled out in several ways. In some experiments (numbers 12, 13 and 14 of Table I) an acid-soaked piece of filter paper was suspended in the experimental vessel, but high respiratory quotients were found throughout most of the period nevertheless. In other experiments, individual flies were confined over acid, which was then tested with Nessler's reagent for the presence of ammonia. A few early trials gave some positive results, but later when more care was taken to exclude contamination from outside sources, even large numbers of flies failed to produce significant amounts of ammonia. The high respiratory quotients were characteristic only of the first three hours or so of measurement, and fell to values of 1 or somewhat less when the observations were carried over longer periods.

While no definite explanation can be given for these facts, which deserve further study, it is suggested that the flies, recently removed from food in the culture bottles, may have been laying down energy reserves by conversion of carbohydrate to fat. Jongbloed and Wiersma (1934) occasionally noted high respiratory quotients before flight in their experiments with bees, but were inclined to ascribe these aberrant values to experimental error.

b. Respiration and rate of wing-beat during flight

Two objectives were in mind during this phase of the experiments: (1) measurement of the respiratory exchange, and (2) a retesting of the correlation found in a previous study between rate of wing-beat and the level of the respiratory metabolism.

As may be seen from the data in Table II, very seldom did the observations indicate a respiratory quotient significantly different from 1, even in the later stages of continuous flights which lasted from 1 to 2 or more hours. Thus the conclusion reached by Williams and colleagues on the basis of glycogen determinations, that carbohydrate constitutes the chief source of the energy required for flight, receives strong support from the present observations. These workers found that 4 to 5 day old *D. funebris* when freshly removed from culture contained glycogen to the extent of 4.88 per cent of the live weight. After 90 minutes of flight only 1.30 per cent remained, so that its rate of disappearance, in terms of the weight of the animal, amounted to about 2.4 per cent per hour. Very similar results were obtained from analyses of thoracic glycogen in the blowfly, *Lucilia sericata*.

For comparison, the weights of glycogen equivalent to the CO_2 which was produced in the flights of the present study have been calculated. It was assumed that complete oxidation of the carbohydrate to CO_2 and water occurred, on which basis the rate of utilization of glycogen during flight amounted to from 2.0 to 3.2 per cent of the final live weight per hour (Table IV). These figures are sufficiently close to those obtained by Williams *et al.* to make it seem likely that glycogen is not merely the principal, but probably the sole source of energy consumed during flight; however, the scatter in the results is such as not to exclude the possibility that smaller amounts of other types of substrate might also have been utilized, although there is no definite evidence pointing in this direction.

An intimate correspondence between the level of activity and the respiratory rate is brought out in the plots of Figure 1, where the logarithms of the rates of CO_2-production are compared with the logarithms of the rates of wing-beat. Each point represents the average rates over a period of approximately 5 minutes, and the rate of production of CO_2 has been corrected by subtraction of the average rate found for the specimen in the preflight resting period.

TABLE IV

Glycogen equivalents of respiration of Drosophila during long flights

Specimen number	Weight	Duration of flight	Total wing-beats (approximate)	Total CO_2 produced		Calculated consumption of glycogen	
	mg.	min.		cu. mm.	micrograms	micrograms	per cent of body weight per hour
1	1.5	87	738,000	56.10	110.0	67.5	3.2
3	0.9	71	573,000	21.79	42.7	26.2	2.4
10	1.2	154	1,268,000	83.20	163.0	100.0	3.2
11	1.4	147	1,064,000	60.00	117.6	72.2	2.1
13	1.5	56	409,000	23.40	46.0	28.2	2.0
14	1.2	64	484,000	31.29	61.5	37.7	2.9

Inspection of the graphs shows clearly that there must be some underlying linear relationship between the logarithms of frequency and respiratory rate. Similar results had been obtained during shorter flights in the majority of the experiments of Chadwick and Gilmour, who reasoned from a comparison of wing movement with simple harmonic motion that the work done per stroke should be proportional to the $(\text{frequency})^2$, so that a direct proportionality between $(\text{frequency})^2$ and the oxygen consumption per stroke would be expected. Essentially the same relationship was developed from a somewhat different approach by Reed *et al.* (1942) in a study in which the characteristic rate of wing-beat under standard conditions was compared with various bodily dimensions for a number of species and races of *Drosophila*. The formula they derived,

$$(\text{wing-beat frequency})^2 = k \times \text{muscle volume}/(\text{wing area})\ (\text{wing length})^3,$$

expresses the fact that the $(\text{wing-beat frequency})^2$ increases in direct proportion to the power of the motor mechanism and in inverse proportion to the resistance to be overcome by the wings.

If the work done per stroke is proportional to the second power of the frequency, then the work done in unit time must be proportional to $(\text{frequency})^3$, and this quantity would be related directly to the rate of respiration, the energy input, through an efficiency factor. In the absence of satisfactory determinations of the work output of *Drosophila* in flight, the actual efficiency remains unknown, but the coefficient may be supposed to include, in addition to the overall efficiency of the biochemical transformations which supply the energy of muscular contraction, a second component expressing the efficiency with which the thorax and wings are able to translate muscular work into movement of air. A perfectly linear relationship between $(\text{frequency})^3$ and rate of respiration would indicate that the efficiency remained absolutely constant throughout the period of flight, or that changes in efficiency were exactly balanced by shifts in amplitude or in the angle of attack.

As an aid in visualizing the actual performance, straight lines have been drawn through each of the plots in Figure 1 to conform to the equation

$$\log\,(CO_2/t - r) + \log k = 3 \log F, \tag{1}$$

where CO_2 = total CO_2 recorded during time t,
r = mean rate of production of CO_2 during the preflight resting period, and
F = average frequency during time t in wing-beats per minute.

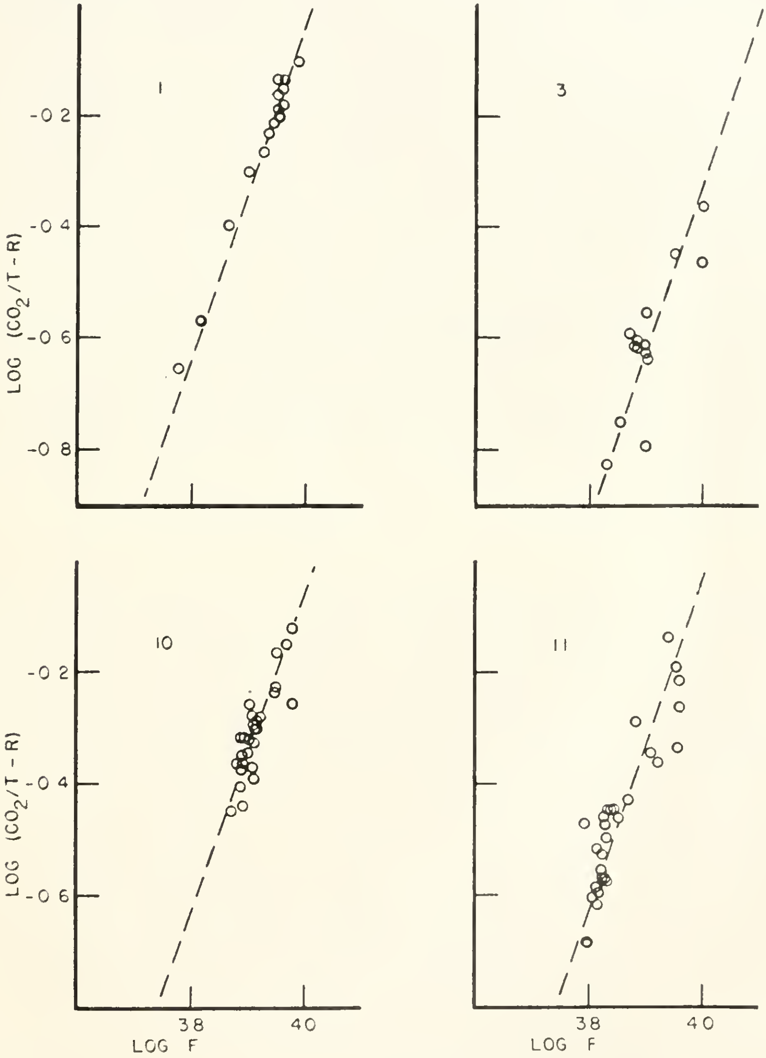

FIGURE 1. CO_2-output as a function of wing frequency in *Drosophila*. Each point represents the average rates during a 5-minute period of flight. The broken lines were calculated to conform to the equation $\log\,(CO_2/t - r) + \log k = 3 \log F$, with values chosen for $\log k$ as follows: Specimen number 1, 12.03; number 3, 12.32; number 10, 12.05; number 11, 12.03.

The quantity k includes a proportionality factor, plus the efficiency, and probably also a component related to the bodily proportions of the individual insect, in accordance with the findings of Reed *et al.* The values of k used for determining the lines of Figure 1 were calculated for each specimen by averaging the values obtained when the paired observations of $(CO_2/t - r)$ and F were substituted in Equation 1.

Although it is evident from the graphs that there is a fairly close correspondence between performance as predicted by Equation 1 and the actual observations, it is clear also that measurements covering a given 5-minute period may depart rather widely from expectation. These random variations are to be explained as due in part to experimental error, the result chiefly of lag effects in absorption of CO_2 when rapid shifts in the rate of respiration occurred, and may be ascribed also in some measure to alterations in the amplitude of the wing stroke and in the angle of attack. Since the work output per minute would vary with the third power of the amplitude and with the sine of the angle of attack, small variations in these quantities would exert an appreciable effect. Such changes are not infrequently noticed under stroboscopic illumination, but unfortunately no satisfactory technique has been developed for their measurement. The absence, in spite of these irregularities, of any consistent trend away from the linear relationship between F^3 and CO_2-output as lower rates are encountered in the later stages of flight is noteworthy, since it shows that in general the efficiency of flight is the same at this time as when the animals were fresh. This could hardly be the case if any great shift had taken place in the biochemical reactions which deliver the energy for flight; thus these observations give added support to the inference already drawn from the respiratory data: that metabolism of carbohydrate furnishes the energy utilized throughout the entire period.

c. Respiration after flight

For the first 5 or 10 minutes following flight the measurements consistently yielded resting rates of respiration much higher than those found before flight (see Table IV). This is almost certainly an artefact resulting from lag in absorption of CO_2 which had been given off during the flight period, and should not be regarded as evidence for an oxygen debt incurred during activity. With smaller vessels in which the lag in absorption was negligible, it was shown previously that the oxygen debt represented an amount sufficient to sustain flight for only a fraction of a minute, that it was independent of the length of flight, and that it was paid off in 2 minutes after flight had ceased.

During subsequent periods rather variable results were obtained, both as regards the respiratory level and the respiratory quotient. Experimental error was magnified at the lower rate of gaseous exchange, and in addition the activity of the specimens varied from time to time. Nevertheless, the values found for the respiratory quotient were often significantly less than 1, although the rate of oxygen consumption fluctuated for some hours within a range close to the preflight average. These observations accord with other evidence that the carbohydrate reserves are seriously depleted by extended flights.

It is obvious, of course, that flight may be, and normally is terminated by causes other than exhaustion of carbohydrate, and since some individuals were found to be able to fly for over 2 hours, there is a fair presumption that specimens whose performance fell short of this figure may have ceased flying with a considerable store of carbohydrate still in reserve. Some of the higher respiratory quotients recorded after flight may be accounted for on this basis. The fact that other individuals survived for a number of hours with respiratory quotients of 0.70 or less points to a fundamental difference between the resting metabolism of *Drosophila* and bees, which according to Beutler succumb within a few minutes if they are allowed to fly until their carbohydrate is exhausted. The survival of these flies presents an opportunity for investigating the process of recovery under controlled feeding which could be made to yield valuable information as to the type of substances which can be converted into sources of energy for flight, a phase of the subject concerning which nothing is known at this time.

SUMMARY

1. Continuous volumetric measurements of oxygen consumption and conductimetric measurements of carbon dioxide production were made at 20° C. on individual specimens of *Drosophila americana* and *D. virilis:* (*a*) before flight; (*b*) during flight to exhaustion; and (*c*) after flight. During flight the rate of wing-beat was determined stroboscopically.

2. Before flight, the average results were: CO_2, 31.7 cu.mm. per gm. per min.; O_2, 26.0 cu.mm. per gm. per min.; R. Q., 1.23.

3. During flights lasting from 56 to 154 minutes the rate of respiration was approximately proportional to (wing-beat frequency)^3. The rate of oxygen consumption averaged 14 times and the rate of CO_2-production 11 times the previous resting rate. The R. Q. was essentially 1.

4. Variable rates of respiration were observed after flight; the R. Q. was frequently much less than 1.

5. It is concluded that carbohydrate furnishes the principal and possibly the only source of energy for flight, and that *Drosophila* are able to survive for some hours after their carbohydrate has been exhausted.

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THE EFFECT OF ILLUMINATION AND STAGE OF TIDE ON THE ATTACHMENT OF BARNACLE CYPRIDS*

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The attachment of barnacle cyprids to exposed surfaces is influenced by the degree of illumination and the surface color. Cyprids of *Balanus amphitrite* and *Balanus improvisus* were found by Visscher (1927) to attach in greater numbers to dark or shadowed surfaces. McDougall (1943) noted this same behavior for the cyprids of *B. eburneus*. When exposed only at night, however, Pomerat and Reiner (1942) noted that *B. eburneus* attached to black, clear and opal glass in equal numbers. Twice as many barnacles attached to the black glass in natural day-night exposures. Gregg (1945) investigated the effect of contrasting dark surfaces against lighter general surroundings. He concluded that while no correlation could be established between the degree of contrast and the frequency of attachment, "shading" was suggested as acting as a stimulus to the attachment of the larvae of *B. eburneus* to opal glass collectors.

In the present investigation the rate of attachment of cyprids of *B. improvisus* during daylight and night exposures, and the effect of artificially illuminating the collecting surface at night were observed. The effect of the tide on the rate of attachment of cyprids was also studied. This work was carried out at the boat shed of the Beach Boat Slips Corporation in Biscayne Bay, Miami Beach, Florida.

METHODS

Four stations for sampling the barnacle cyprid population were established under and adjacent to a covered boat slip which extended 250 feet from the shore, the stations being at the outer end of the shed.

Stations A, B and C were control stations for comparison with Station D, which was illuminated at night. Stations A, D and B were spaced 55 feet apart, in that order in a north-south line paralleling the general flood and ebb movement of the tidal current. Station C was located 60 feet to the west of Station D. The conditions of natural and artificial illumination at each of these stations were as follows: In the daylight hours illumination at each of the four stations was nearly the same through the day. The intensity at each location depended on the proximity of the shadow of the shed. At Station A, daylight illumination was skylight; at Station B, the collector swung in and out of the sun and shadow of the shed with the phase of the tide; and Station C was in full sunlight, whereas Station D was under the shed roof and in the full shadow. At night, Station D was artificially illuminated, while Stations A, B and C were in darkness.

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* Contribution No. 400 from the Woods Hole Oceanographic Institution. The observations were made during an investigation by the Woods Hole Oceanographic Institution under contract with the Bureau of Ships, Navy Department, which has given permission for their publication. The opinions presented here are those of the author and do not necessarily reflect the official opinion of the Navy Department or naval service at large. The author is indebted to Dr. B. H. Ketchum, Dr. L. W. Hutchins and Dr. A. C. Redfield for helpful advice and criticism and to Mr. Alexander C. Frue for assistance in making the observations.

Collecting units consisting of four pieces of black glass (500 sq. cm. collecting area) were suspended from a float. The surface panel was horizontal while the others, at depths of 2, 6 and 10 feet, were hung in a vertical position. The 10-foot level was just above the bottom at low tide and three feet higher at high tide.

The periods of collection, day and night, were eleven hours each, from 7:30 A.M.–6:30 P.M., and 7:30 P.M.–6:30 A.M. The eleven-hour exposure permitted no significant overlapping period of dark to light or light to dark when the collectors were in the sea. The one-hour interval between exposures permitted counting, cleaning and reimmersing of the collection units.

TABLE I

Numbers of cyprids attaching during consecutive night and day periods of eleven hours each at three stations. The figures are the sum of the collections on four panels of 500 sq. cm. area each, hung at different depths.

Cyprid attachment night			Cyprid attachment day			Average	
Stations			Stations			Night	Day
A	B	C	A	B	C		
*394	205	306	403	614	784	302	600
418	279	272	386	897	997	323	760
463	534	778	1361	1392	2018	592	1590
434	768	497	701	666	1147	566	838
296	160	483				313	
**444	533	432	2352	1149	3564	470	2355
391	446	541	636	569	1829	459	1011
255	201	479	432	881	1092	312	802
219	168	240	676	564	1581	209	940
114	99	101				105	

* February 11, 1946–February 16, 1946. ** February 26, 1946–March 3, 1946.

Although the source of illumination at Station D remained fixed while the collector unit which hung below it rose and fell with the tide, an average distance of 5 feet from the lights to the water surface was used in calculating the value of incident illumination at the water surface and at the 10-foot depth. The incident illumination five feet from the lights was measured by a direct reading photometer and that at the 10-foot level calculated by using an absorption factor for the local waters determined with an underwater photometer.

The study of cyprid attachment relative to stage and direction of the tide was made by counting the cyprids attaching during intervals of two hours throughout a tidal cycle in daylight hours only. The collecting units were the same as those employed in the illumination experiments.

Diurnal Variation in Cyprid Attachment

Observations on five successive nights and four intervening days in two separate series indicated that many more cyprids settled during the period of daylight, as shown in Table I. In the first set of observations, the ratio of the average of all the day collections to the average of the corresponding night collections indicated an increase of 2.1 times the number of cyprids attaching in daylight, while in the second series the ratio of day to night attachments was 3.5.

It is of interest to note that in the first set of collections when the cyprid density, as shown by total attachment day and night, was lower than the second set of collections there are examples of day collections being nearly of the same magnitude or even slightly less than the night attachments. This condition was found only at Stations A and B which were wholly or partially shaded from direct sunlight during the daylight hours. A partial explanation of the relatively high night attachments of the first set of collections may be due to the fact that these were made under the full of the moon whereas the second set were made in the dark of the moon. However, when the cyprid density was high, as during the second set of observations, no discrepancies from the diurnal fluctuation in attachment were noted. The maximum differences between night and day attachments were always found at Station C which had the maximum difference in illumination.

Vertical Distribution of Cyprid Attachment

The vertical distribution of barnacle cyprids at Stations A, B and C showed no consistent pattern which would suggest dependence on any particular controlling factor. In the first set of collections, shown in Figure 1, the maximum numbers at Station A, both day and night, were found at the 2-foot level. At Station B, all the day collections showed cyprid peaks at the 6-foot level, whereas, at night, attachment was nearly uniform between the 2-foot level and the bottom, except for one observation. At Station C, the day collections resulted in maxima at the 2-foot level on three occasions and a 6-foot maximum on one occasion.

The second series of vertical samplings, as shown in Figure 2, were made at a period of higher cyprid density which resulted in collections of considerable magnitude for several of the eleven-hour periods. However, no consistent pattern of vertical distribution was apparent.

This erratic distribution of cyprids, both vertically and in comparison of stations, is of particular interest, in view of the relatively close proximity of the collecting units. The apparent random attachment of cyprids to the collecting units is probably due to a nonuniform distribution of cyprids in the water. Since the number of cyprids attaching does not decrease with depth, it is apparent that intensity of illumination does not control vertical distribution.

Effect of Artificial Illumination on Cyprid Attachment

The normal diurnal cycle, with lower attachment numbers at night, was abolished by the use of artificial illumination at night at Station D. The cyprid attachments at night under artificial illumination at Station D were greater than the daylight counts at this location and were nearly as large as the daytime collections at Station C where the intensity of daylight illumination was greatest. As shown in

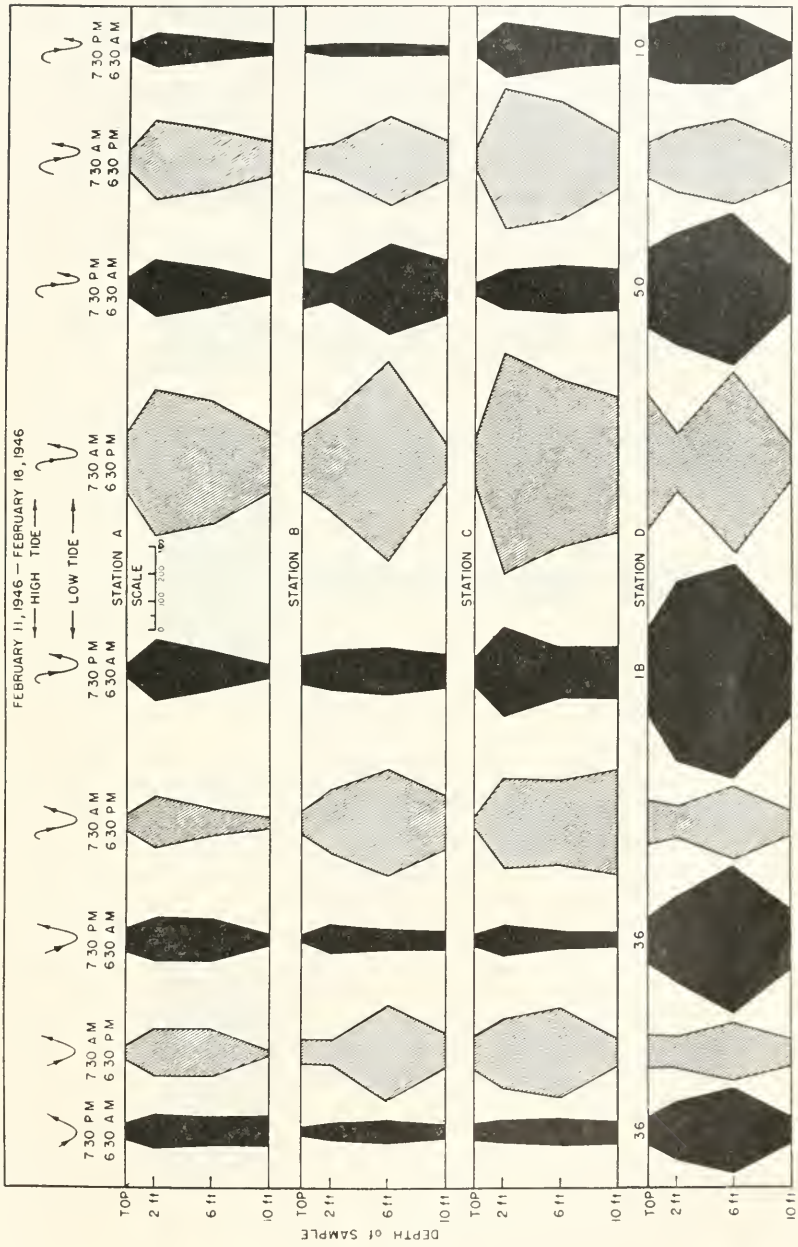

FIGURE 1. Vertical distribution of cyprids at four stations as collected in eleven-hour periods, February 11, 1946–February 16, 1946. The tidal curves at the top of the figure represent the stages of the tide in which the collection took place. The numbers over the night collections of Station D are the illumination values in footcandles at the water surface used for that exposure.

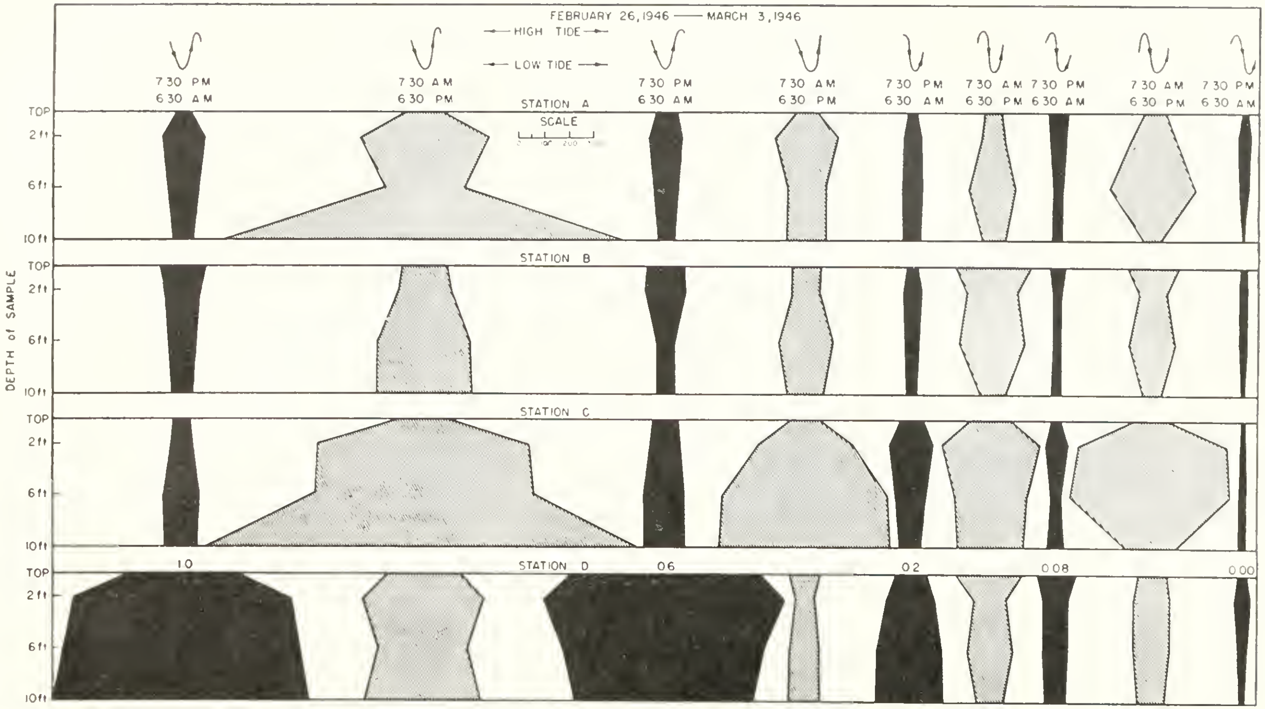

FIGURE 2. Vertical distribution of cyprids at four stations as collected in eleven-hour periods, February 26, 1946–March 3, 1946. The tidal curves at the top of the figure represent the stages of the tide in which the collection took place. The numbers over the night collections of Station D are the illumination values in footcandles at the water surface used for that exposure.

Figures 1 and 2, artificial illumination, however, had little if any effect on vertical distribution of the cyprids.

In order to determine the intensity of illumination necessary to increase the cyprid attachments at night, the illumination over the collectors at Station D was systematically changed. In the first series, the incident illumination at the water surface was varied from 36–1.0 footcandles, and in the second experiment, from 1.0–0.00 footcandles. The variations in vertical distribution at each station, which are apparently due to other factors, have been smoothed by totaling the cyprid accu-

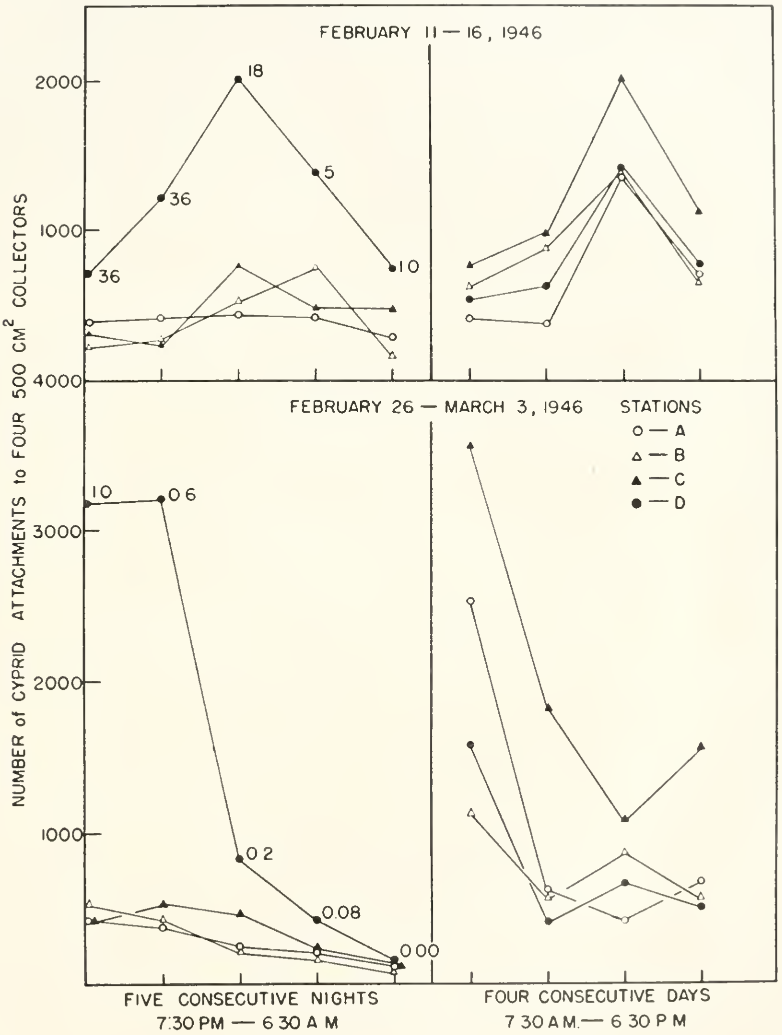

FIGURE 3. Total cyprid attachment for the eleven-hour collection periods at each station. The numbers plotted with Station D, night collections, are the values in footcandles at the water surface of the artificial illumination employed.

mulations from each of the four glass collectors. This figure then represents the catch from the cyprid population of a vertical column of water. As shown in Figure 3, the illumination of the collectors at Station D resulted in a two to sevenfold increase in numbers of cyprids attaching over those found on the control surfaces. The daily variation in cyprid numbers attaching at night to the illuminated collectors of Station D resembled more nearly the daily fluctuation in cyprid density as found on the daylight collections of Station C. Although the night collections of Station D paralleled the cyprid density fluctuations as found at the unilluminated stations, they were of course several times greater in magnitude. This result was obtained within the limits of illumination of 36 to .08 footcandles at the water surface. On the final night exposure of the second series, when Station D was not illuminated, its cyprid collection was still slightly greater than the other three stations. However, this difference is probably within the normal variation. During the day the collections at Station D were of the same magnitude as at A and B and considerably less than those at C.

TABLE II

Numbers of cyprids attaching in eleven hours at night at the 10-foot depth at Station D as related to quantity of artificial illumination and in comparison with the simultaneous attachment at Stations A, B and C.

Value of illumination at D at 10-foot depth (footcandles)	Stations				$\frac{D}{1/3(A+B+C)}$
	A	B	C	D	
*5.2	110	45	87	102	1.3
5.2	38	71	56	219	4.0
2.5	45	117	184	318	2.8
.71	42	206	136	169	1.3
.14	44	34	90	64	1.1
** .14	78	92	135	1005	9.9
.09	59	71	158	710	7.4
.03	65	37	81	257	4.2
.001	38	44	66	77	1.6
.000	7	15	33	21	1.1

* Five consecutive nights—February 11, 1946–February 16, 1946.
** Five consecutive nights—February 26, 1946–March 3, 1946.

The artificial illumination, even at the 10-foot depth, consistently increased the number of cyprids attaching to the collecting surface of Station D in comparison with the control stations, as shown in Table II. The ratio of the attachment at Station D to the averages of Stations A, B and C indicates the degree of change in attachment due to illumination. The greatest differences between the illuminated collectors and controls occurred on the second and third nights of the first experiment when cyprid attachment was at a maximum. In the second set of exposures, when cyprid density was high, all the collections at the 10-foot level of Station D, even though the illumination was very low, were invariably greater than all the controls.

As indicated above, the illumination of the water surrounding the collectors was varied systematically to determine the minimum value to which the cyprid would respond. When the cyprid density was high, thus providing more organisms which would by random selection be subjected to the contrast of luminous zone and dark area, the greatest change in cyprid attachment occurred when the illumination of the water surface was changed from 0.6 to 0.2 footcandle. At 0.6 footcandle and greater intensities, the difference in cyprid settling, in comparison to the controls, was sevenfold, while at 0.2 footcandle and less, the increase over the control collectors was only twofold. However, at illumination as low as .03 footcandle, increases in attachment over control collectors were still considerable.

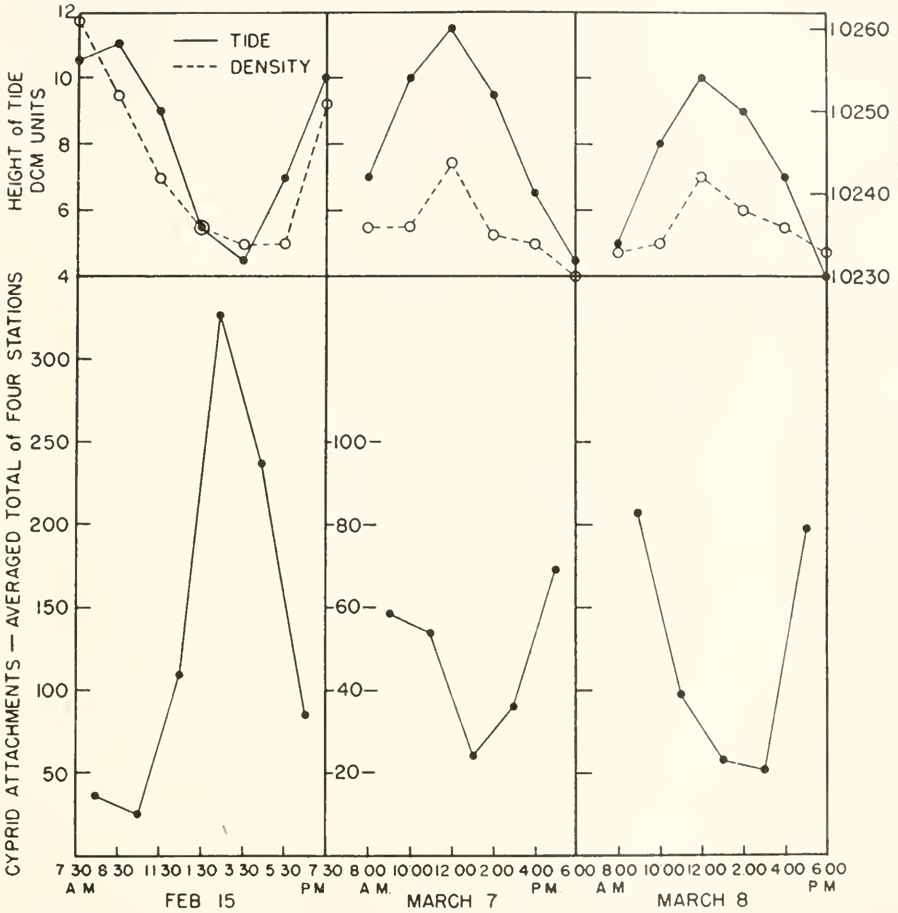

FIGURE 4. Bi-hourly cyprid attachment at each station totaled from the four collecting surfaces. The height of tide is noted in decimeter units. February 15, March 7 and 8, 1946.

Previous investigations (Visscher, 1927; Pomerat and Reiner, 1942; and McDougall, 1943) showed that the barnacle cyprid attaches in daylight in greater numbers to dark surfaces than to light surfaces. At night, as is to be expected, there is no distinction between the two colors. In this investigation illumination of dark collecting surfaces at night increased attachment. One effect of illumination is to increase the contrast between the dark surfaces and the surrounding water. In waters with considerable suspended material, as usually found in harbors and estuaries, a beam of light is markedly scattered, producing a general illumination in the water. A piece of black glass suspended in these waters under a source of illumination was discernible to the observer as a black area surrounded by brighter water. When the water appears brighter than the collecting surface, at night because of artificial illumination, or during daylight, the cyprids may be attracted to the darker area by some negative phototropic response and may accumulate there in greater numbers than would result from chance encounters. In contrast, in the absence of light at night, the numbers of cyprids attaching may depend solely on chance encounters with the collecting surface, and are consequently lower than during periods of illumination.

Effect of Stage of Tide

On collectors exposed through the day and examined at two-hour intervals, the maximum numbers of cyprids attached during the low-tide period as shown in Figure 4. This was found whether the low tide occurred at midday, in the early morning, or late afternoon. As in the case of the longer exposures, there was no indication that the distribution of cyprids on the panels was influenced by the depth at which they were hung.

At the location of this experiment, the ebbing tide brings to the boatshed water which is isolated within the bay. It is replaced at high tide with oceanic water entering the bay on the flood. This is shown by the change in density accompanying the tidal cycle. The isolation of the bay water permits development of a large cyprid population, since few of the plankton stages are lost into the ocean prior to metamorphosis to the cyprid stage. At the low-tide period, the sampling location was occupied by the bay water with high cyprid content, and this apparently accounts for the greater attachment at that time. The high attachment at low tide cannot depend upon the lessened flow at that time, since a similar slack water occurs at high tide when the attachment is minimal.

Summary

1. The cyprid larvae of *Balanus improvisus* were found to settle in a diurnal rhythm with maximum numbers attaching during daylight hours.

2. No consistent pattern of vertical distribution of the cyprids was found.

3. The normal diurnal cycle in rate of attachment of barnacle cyprids was nullified by the use of artificial illumination over the collecting surfaces at night.

4. The magnitude of the cyprid collection on the artificially illuminated surfaces was equal to the collection on the sun-illuminated surfaces in daylight.

5. The intensity of artificial light necessary to produce large cyprid attachments at night was of an order as low as 1 footcandle at the water surface.

6. No correlation was found between the quantity of artificial light at night and the numbers of cyprids attached.

7. The highest rate of cyprid attachment relative to the phase of the tide was found to occur when the waters of upper Biscayne Bay were sampled at the collecting station. This body of water reached the sampling station at low tide and was characterized by a high cyprid population.

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EARLY LIFE HISTORY OF THE OYSTER CRAB, PINNOTHERES OSTREUM (SAY)[1]

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Introduction

The first and second zoeae of *Pinnotheres ostreum* have been described by Hyman (1924). The five crab stages parasitic in the oyster (*Ostrea virginica*) have been described by Stauber (1945). The present paper is an attempt to complete the knowledge of the development of the oyster crab from the egg to the first crab instar. This work was done at the Virginia Fisheries Laboratory in the summer of 1944, under the direction of Dr. Curtis L. Newcombe.

The first Pinnotherid larva to be described was the zoea of *Pinnotheres pisum* Leach, the British pea crab. This species has three or more zoeae. Other members of the family are said to have two, three or four zoeal stages; in some species a megalops stage has been described, while one at least is said to pass directly from the last zoea to the first crab. The family Pinnotheridae is noted for the great diversity of the zoeae in different species, and this lack of uniformity seems to apply also to the number of larval stages. Faxon (1879) stated that the last zoea of *Pinnixa chaetopterana* molted directly into a first crab stage, without a megalops stage. Smith (1880) found a megalops in another species of Pinnixa. There are only two published descriptions of Pinnotherid megalops: *Pinnotheres veterum* by Lebour (1928) and *Pinnotheres taylori* by Hart (1935). In the latter there are two zoeae and one megalops.

The first zoea of *Pinnotheres ostreum* was hatched from the egg by Birge (1878), but he was unable to rear second zoeae. Hyman (1925) obtained the first zoeae of *Dissodactylus mettitae, Pinnotheres maculatus*, and *P. ostreum* by hatching, and described the second zoea of *P. ostreum* from specimens reared from first zoeae caught in plankton.

In the present study, all specimens were reared in the laboratory from the egg. *Pinnotheres ostreum* was found to have four zoeal stages and one megalops, followed by the first crab.

Methods

Ovigerous female crabs were removed from oysters and the egg strands were cut away and placed in shallow enamel pans containing sea water. The salinity of the water used varied from 20 to 26 parts per thousand while the temperature was approximately 23° C.

On July 15, 1944, a medium brown sponge was removed from an oyster crab and placed in water. One week later first zoeae emerged from the eggs. Ecdysis of the larval cuticle occurred simultaneously with hatching. Five days later some first zoeae molted to become second zoeae, and three days afterward third zoeae appeared. All of these third zoeae died in ecdysis without becoming fourth zoeae.

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[1] Contribution from the Virginia Fisheries Laboratory of the College of William and Mary and Commission of Fisheries of Virginia, Number 27.

A second experiment was begun on July 16, 1944. A younger sponge of bright orange color was placed in hatching pans. First zoeae hatched at the end of twelve days and after three days these specimens became second zoeae. In an additional five days the third instar appeared, and after seven more days several individuals reached the fourth zoeal stage. There was a high mortality rate during this ecdysis. The fourth zoeae were placed in individual glass finger bowls. Six days later one megalops completed a successful ecdysis and became a first crab. Three other fourth zoeae also reached the megalops stage and eventually one of these became a first crab, but the times were not noted. The total time in this experiment, from the recently laid orange eggs to the megalops stage, was 33 days, and the total time required to obtain the first crab was 38 days. The period of larval development, from hatching to first crab, was 25 days.

The length of time spent in each instar during the development seemed to depend on a number of factors, food abundance and water temperature having the most obvious effect. Few complete records on embryonic and larval development of crabs have been published. Hart (1935) found that four or five weeks were required for *Pinnotheres taylori* to pass through two zoeal instars and one megalops stage.

The food used in rearing *P. ostreum* larvae consisted of concentrated plankton from York River. A yellow dinoflagellate was eaten most readily by the zoeae, but they also fed on crab eggs. Excess food was removed after feeding to prevent fouling. Water was changed every second day and was aerated several times daily by pipetting.

In the morphological study of life history stages it was necessary to use the material conservatively, since very few specimens of each stage were preserved. Killing and preservation were in 5 per cent formalin. For study, each specimen was placed in a drop of glycerine on a slide, a cover glass was put on, and the specimen was drawn *in toto* without flattening. Megalops and first crab stages were placed on depression slides to avoid distortion. Cover glasses were removed after toto drawings had been made, and the specimens were dissected with ordinary dissecting needles. Appendages were pulled off, not cut. Dissected parts were arranged in the glycerine with needles and the cover glass replaced, with slight pressure. No great difficulty was experienced with this method; in nearly every case at least one member of each pair of appendages was found intact and in position to draw. All drawings were made with a camera lucida; only very fine details such as the setules on feathered setae were added free hand.

Description of Stages

First zoea (Figs. 1, 3, 4, 6, 8, 9, 12)

In living specimens, fully extended but not flattened, the total length is 0.90 mm. In formalin-preserved specimens the total length is 0.7 to 0.9 mm. The carapace averages 0.42 mm. in length and 0.27 mm. in width. The eyes are 0.11 to 0.13 mm. in diameter. There are no dorsal or lateral spines on the carapace.

The abdomen has five segments, not counting the telson; its width increases toward the posterior end. The second and third abdominal somite bear short triangular lateral knobs. The telson has three toothlike lobes, with three setae on each side between the lateral and median lobe. The chromatophores are distributed as described by Hyman (1925). The antennules are folded to a spherical form, and bear two large and one small aesthete or sensory hair. The antennae are not visible. The first maxilla (maxillule) has no "epipodital hair"; the protopodite bears a chromatophore; the proximal (coxopodite) lobe bears four setae, the distal (basipodite) lobe has five, and there are four setae arranged in two groups of two each on the two-segmented endopodite. The second maxilla has a chromatophore on the protopodite, five setae on the coxopodite, eight or nine on the basipodite, three on the endopodite, and four on the scaphognathite. The first maxilliped has chromatophores in the coxopodite and basipodite; the basipodite bears eight setae, the exopodite has four long terminal setae or "swimming hairs," and the five-segmented endopodite has one seta on the proximal segment, two on the second, one on the third, two on the fourth, and five on the terminal segment of which four are terminal. The second maxilliped has a chromatophore on the coxopodite, a chromatophore and four setae on the basipodite, four "swimming hairs" on the exopodite, and four setae on the terminal segment of the two-segmented endopodite. No buds of other appendages are visible.

Second zoea (Figs. 2, 10, 13).

The total length of preserved specimens is 1.48 mm., but these specimens have an abnormal swelling between the thorax and abdomen; the normal length would be somewhat less. The length of the carapace is 0.57 mm. The eye is 0.12 to 0.14 mm. in diameter. The first maxilla has an external seta or "epipodital hair," and there are five, seven, and four setae on the coxopodite, basipodite and endopodite, respectively. The second maxilla has six, nine, and three setae on these three endites; the scaphognathite has three apical setae and five near the basal end. The

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EXPANATION OF PLATE I

All drawings made with camera lucida from specimens killed and preserved in 5 per cent formalin, mounted in glycerine. Scale A represents 0.4 mm. in Figures 1, 2, 6, 7, 7a, and 8. Scale B represents 0.1 mm. in Figures 3 and 4.

FIGURE 1. First zoea of *Pinnotheres ostreum*, hatched from egg in laboratory, drawn with 10 × 10 × lenses.

FIGURE 2. Second zoea of *P. ostreum* reared from egg in laboratory, drawn with 10 × 10 × lenses.

FIGURE 3. First maxilliped of first zoea, *P. ostreum*, drawn with 7.5 × 43 × lenses; swimming hairs of exopodite cut off to allow placement of figure on plate.

FIGURE 4. Second maxilliped of first zoea, *P. ostreum*, drawn with 7.5 × 43 × lenses; swimming hairs cut off figure.

FIGURE 5. Telson of third zoea, *P. ostreum*, drawn with 15 × 10 × lenses.

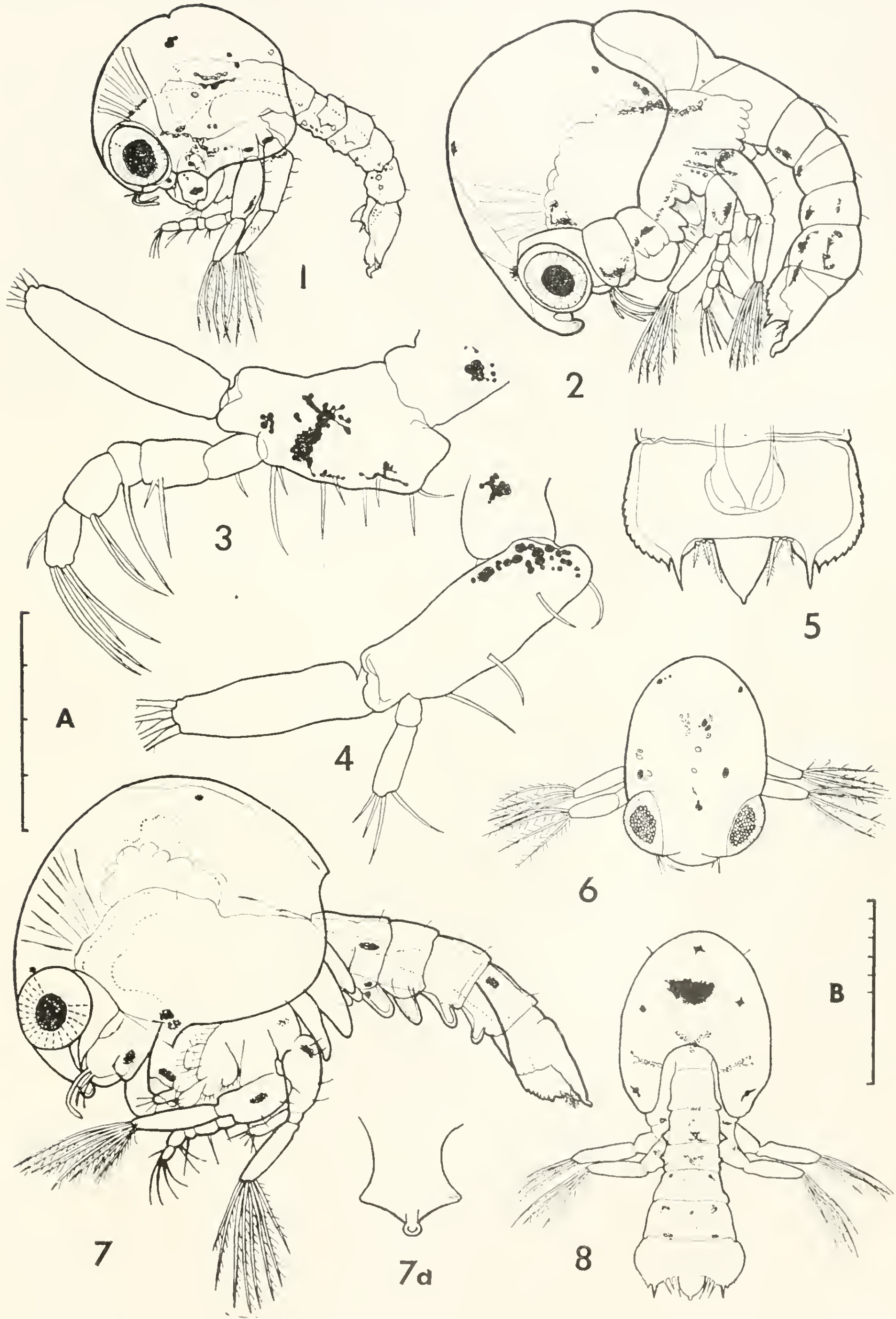
FIGURE 6. Anterior view of first zoea, *P. ostreum*, drawn with 10 × 10 × lenses.

FIGURE 7. Third zoea of *P. ostreum* reared from egg in laboratory, drawn with 10 × 10 × lenses.

FIGURE 7a. Rostrum of third zoea, *P. ostreum*, frontal view, drawn with 10 × 10 × lenses.

FIGURE 8. Posterior view of first zoea, *P. ostreum*, showing dorsal view of abdomen, drawn with 10 × 10 × lenses.

PLATE I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2
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A
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7a
8
B

first and second maxillipeds each have six swimming hairs on the exopodite. Buds of other thoracic appendages can be seen, but there are still no buds on the abdomen. Other features are as in the first zoea.

Third zoea (Figures 5, 7, 7a, 11, 14)

The total length of preserved specimens is 1.3 mm. The carapace is 0.60 mm. long (0.58 to 0.63). The eye is 0.14 to 0.16 mm. in diameter. The antennule bears three large and one small aesthete. The first maxilla has five setae on the coxopodite, eight or nine on the basipodite, and four on the endopodite. The second maxilla has seven to nine setae on the coxopodite, nine or ten on the basipodite, and three on the endopodite; the scaphognathite has five apical setae and eight to thirteen setae between the proximal end and the apical setae. The coxopodite of the first maxilliped has three setae, the basipodite has eight or nine setae, and the exopodite has eight swimming hairs. The exopodite of the second maxilliped also has eight swimming hairs. Buds of the other thoracic appendages are prominent and buds of the abdominal appendages are visible. Other features are as in the first zoea, except for size.

Fourth zoea

Only four or five specimens were obtained, and they molted to become megalops before they could be studied. The only known feature is the possession of ten swimming hairs on each of the maxillipeds.

Megalops (Figs. 15, 16, 17, 18)

The total length is 1.0 to 1.05 mm. The carapace is 0.60 mm. long and 0.58 mm. wide. The abdomen, extended, is 0.40 to 0.45 mm. long and 0.17 mm. wide across the second segment. The eye is about 0.14 mm. in diameter. The carapace has no spines, on the rostrum or elsewhere, but has four to nine setae along each lateral edge. The fifth leg has no "feelers" on the last segment. The antennule has seven or eight aesthetes on the distal segment (outer flagellum). The antenna

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Explanation of Plate II

All drawings made with camera lucida from specimens killed and preserved in 5 per cent formalin, mounted in glycerine. Scale A represents 0.4 mm. in Figures 15 and 18. Scale B represents 0.1 mm. in Figures 9, 10, 11, 12, 13, and 14. Scale C represents 0.1 mm. in Figures 16 and 17.

Figure 9. First maxilla of first zoea, *Pinnotheres ostreum*, drawn with 7.5 × 43 × lenses.

Figure 10. First maxilla of second zoea, *P. ostreum*, drawn with 7.5 × 43 × lenses.

Figure 11. First maxilla of third zoea, *P. ostreum*, drawn with 7.5 × 43 × lenses.

Figure 12. Second maxilla of first zoea, *P. ostreum*, drawn with 7.5 × 43 × lenses.

Figure 13. Second maxilla of second zoea, *P. ostreum*, drawn with 7.5 × 43 × lenses.

Figure 14. Second maxilla of third zoea, *P. ostreum*, drawn with 7.5 × 43 × lenses.

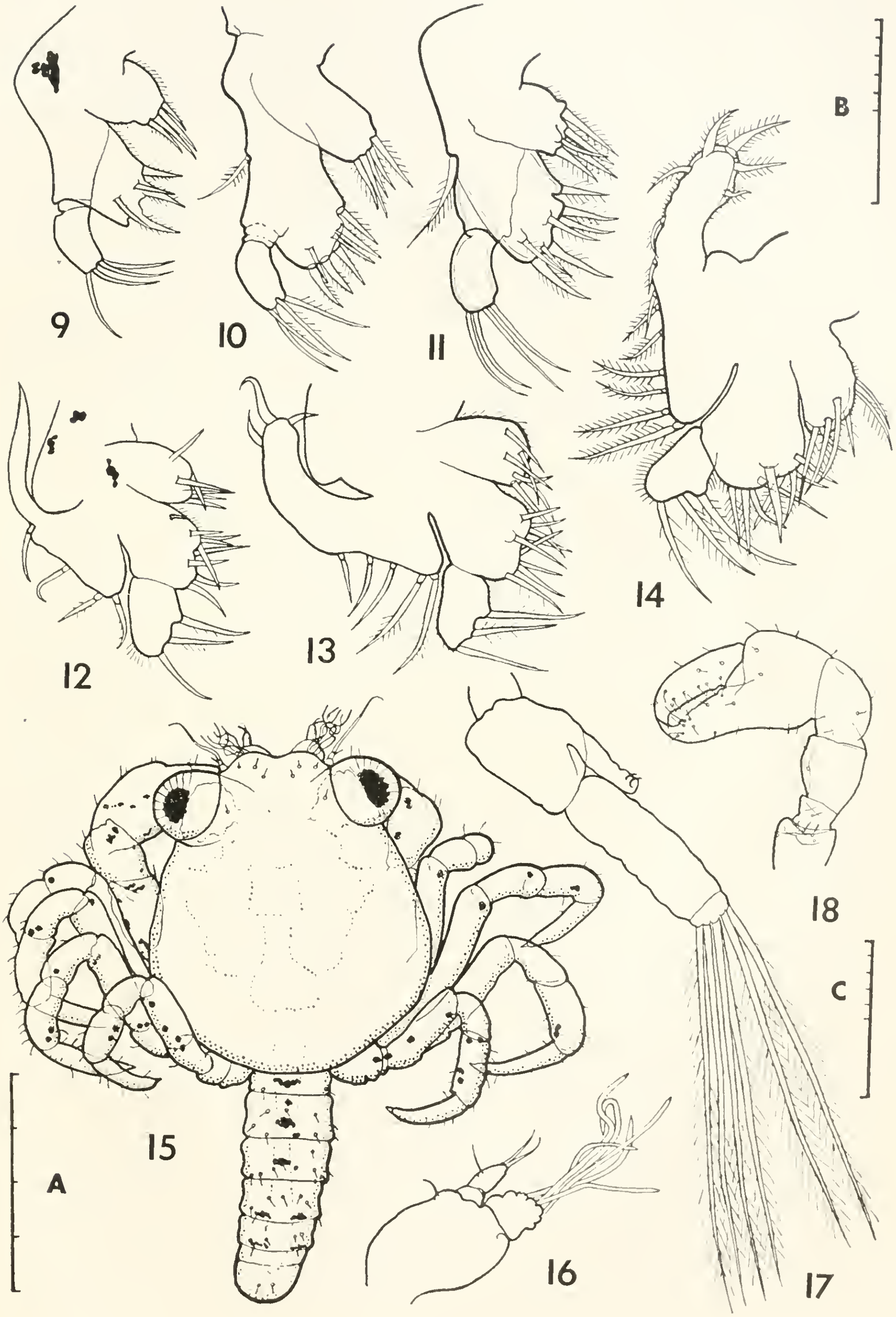
Figure 15. Megalops of *P. ostreum* reared from egg in laboratory, dorsal view, drawn with 10 × 10 × lenses.

Figure 16. Distal portion of antennule of megalops, *P. ostreum*, drawn with 6 × 44 × lenses.

Figure 17. First pleopod of megalops, *P. ostreum*, drawn with 6 × 44 × lenses.

Figure 18. Dorsal view of right cheliped of megalops, *P. ostreum*, drawn with 10 × 10 × lenses.

PLATE II


9
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is five-segmented with a long slender process and a short seta on the distal segment. The abdomen seems to have six segments, but the sixth segment is very indistinct and may be fused with the telson. There are four pairs of pleopods, on the second, third, fourth and fifth segments; the exopods of the first three pairs bear six swimming hairs; those of the last pair have only five. (In the other specimen, the first pleopods have five hairs, the second and third have six, and the fourth have four.)

First crab (Figs. 19, 20, 21, 22, 23)

The carapace is 0.61 mm. long and 0.59 mm. wide. The diameter of the eye is about 0.14 mm. There are no spines on the carapace, but each lateral edge bears seven to nine setae. The last two segments of the third and fourth legs bear long plumose setae, but there are no "feelers" on the fifth leg.

It should be noted that these two laboratory-reared specimens are considerably smaller than the "first stage females" of Stauber (1945). Stauber used this name for the youngest females which invade the oyster, evidently not intending to imply that they were in the first crab instar. Probably there are two or more free-living crab instars before the invasive stage. The smallest "first stage females" found in oysters by Stauber were 1.4 mm. wide, and the smallest males were 1.5 mm.

Oyster crabs in Virginia oysters

No systematic study of the distribution, abundance, and effects of oyster crabs in Virginia has been made, but field notes accumulated by the Virginia Fisheries Laboratory give some data on these points. *Pinnotheres ostreum* has been found on all Virginia oyster grounds which have been observed. The percentage of oysters infested, on different oyster rocks, varies from less than 1 per cent to over 80 per cent, averaging around 30 or 40 per cent. Immature and mature female oyster crabs are found singly in Virginia oysters throughout the year. In only 5 of 276 infested oysters examined in 1943–44 were two crabs found in one oyster. Multiple infestations by male and female early-stage crabs, such as were described in New Jersey by Stauber (1942, 1945), were found only once in Virginia, at Cedar Island, James River, in the summer of 1945. Notes on females bearing sponges extend only from June through August, but the actual spawning season is probably much longer than this. *P. ostreum* zoeae have been found in plankton tows from June through August, but few tows were made in other months.

Significant damage to the gills of oysters by oyster crabs has been noted in many cases. Field notes on the condition of oysters generally show poorer condition in

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EXPLANATION OF PLATE III

All drawings made with camera lucida from specimens killed and preserved in 5 per cent formalin, mounted in glycerine. Scale A represents 0.4 mm. in Figures 20, 22, and 23. Scale C represents 0.1 mm. in Figures 19 and 21.

FIGURE 19. Antenna of first crab instar, *P. ostreum*, drawn with 6 × 44 × lenses.

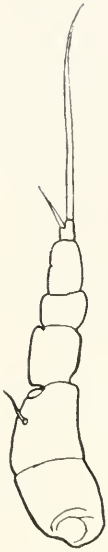
FIGURE 20. First crab instar, *P. ostreum*, reared in laboratory, dorsal view, pigment omitted, drawn with 10 × 10 × lenses.

FIGURE 21. Antennule of first crab instar, *P. ostreum*, drawn with 6 × 44 × lenses.

FIGURE 22. Right cheliped of first crab instar, *P. ostreum*, dorsal view, drawn with 10 × 10 × lenses.

FIGURE 23. First crab instar of *P. ostreum* reared from egg in laboratory, ventral view, drawn with 10 × 10 × lenses.

PLATE III



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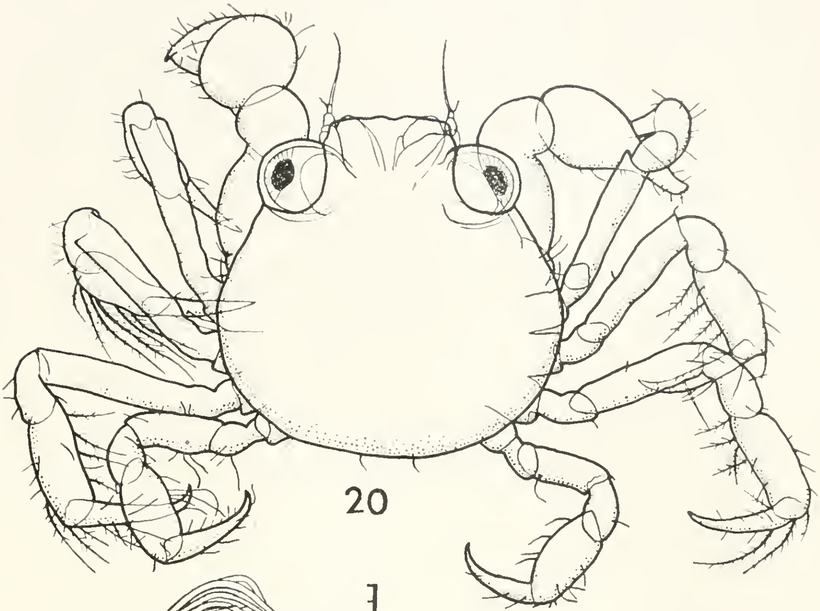

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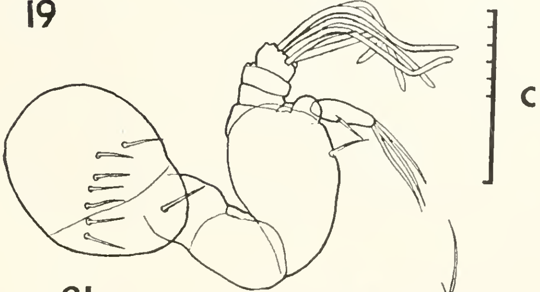

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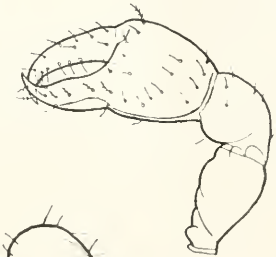

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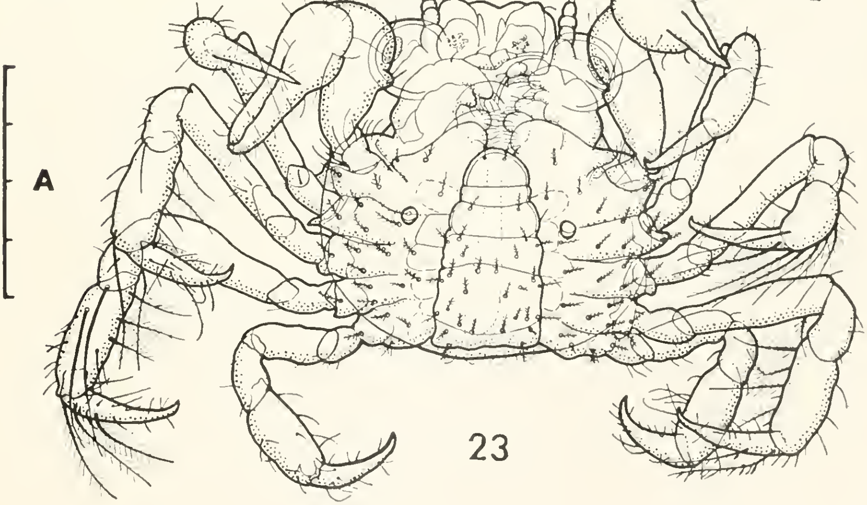

23

crab-infested than in uninfested oysters, but there are often some crab-infested oysters which show excellent condition. Miss Alice Elizabeth Overcash, in an unpublished thesis (1946) submitted to the College of William and Mary, has studied the "index of condition" of Virginia oysters, including many with oyster crabs. She reports that this index $\frac{(1000 \times \text{dry weight of meat in grams})}{(\text{Volume of shell cavity in milliliters})}$ averaged only 82.3 for crab-infested oysters in York River, compared to 90.6 for oysters without crabs. In Rappahannock River she found the mean index of oysters with crabs to be only 71.7, while the entire sample averaged 90.0. Both samples showed significantly poorer condition in infested oysters.

There seems to be no doubt that oyster crabs do injure oysters to some extent, but we have no evidence that they have ever caused the death of oysters in Virginia, as they did in the New Jersey outbreak reported by Stauber (1942, 1945).

SUMMARY

1. The early stages of *Pinnotheres ostreum* have been reared in the laboratory from the egg to the first crab.

2. There are four zoeal stages and one megalops stage. The four zoeal instars are distinguished by having 4, 6, 8, and 10 swimming hairs on the exopodites of both maxillipeds.

3. The time required for development from the early egg (from orange-colored egg mass) to the first crab instar was 38 days. From hatching to the first crab required 25 days.

4. The first crab being only 0.6 mm. wide, at least one more instar must intervene before *P. ostreum* reaches the "first stage" described by Stauber as the youngest crabs which invade the oyster.

5. Oyster crabs are widely distributed and abundant in Virginia waters. They tend to keep oyster meats in relatively poor condition, but have never been observed to cause mortality of oysters in Virginia.

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ELECTROKINETIC STUDIES OF MARINE OVA. V. EFFECT OF PH-CHANGES ON THE SURFACE POTENTIALS OF SEA-URCHIN EGGS

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In a preliminary account (Dan, 1931), it was reported that the cataphoretic potentials of the unfertilized eggs of the sea-urchin, *Arbacia punctulata,* could not be reversed in sign on raising the concentration of hydrogen ions of the surrounding medium. The present paper reports experiments of a similar type which were repeated on other kinds of sea-urchins, *Anthocidaris crassispina* and *Pseudocentrotus depressus,* and the study was further extended to include fertilized eggs.

METHOD

Removal of the jelly. When the unfertilized surface was studied, unless otherwise stated, the jelly was removed by acid. While a dense suspension of eggs was being gently shaken, 1/10 N HCl was added drop by drop until the eggs began to show the first sign of sticking to the glass container. After a few more gentle shakings, a large quantity of sea water was added and the eggs were washed thoroughly by decantation. By this means, it was possible to reduce the time of the eggs' coming in contact with the concentrated acid to the minimum (less than 10 seconds).

pH adjustment. HCl was added to filtered sea water to an amount ample to break down the bicarbonate buffer system. After equilibration, more acid or alkali was added to adjust the pH value to the desired level by indicators. When the desired pH levels were near neutrality, 1/10 N acid or alkali was used, but when they were far removed from neutrality, ½ N solutions were added to minimize the lowering of the osmotic pressures. A slight lowering of the osmotic pressure by using 1/10 N within the limit here encountered does not affect the surface potentials of sea-urchin eggs (Dan, 1936). During the experimentation, the supernatant solution of the egg suspension was frequently checked for pH value to insure its constancy.

In very acidic media, the eggs were injured. At pH 3.5 or 4.0 they could still keep a fairly high fertilizability, if they were left unstirred in the acid and later fertilized after being returned to the normal medium. However, in the present experiment, stirring was technically unavoidable to some extent. In spite of this, pH values down to 2.0 were used for the purpose of obtaining a sufficient insight into the electrokinetic property of the egg surface, taking advantage of the fact that the killing of the eggs does not modify the potentials (Dan, 1934; 1936; see also Winslow, Falk and Caulfield, 1923; Abramson, 1929).

Measurement of the potential. The method of measuring the cataphoretic potentials was practically the same as was reported in the preliminary note and more fully discussed in the first paper of this series (Dan, 1933). This was to use a horizontal microscope on which a Northrop-Kunitz chamber was mounted. Since the stage of the horizontal microscope was situated vertically, the short axis of the cataphoretic chamber came to lie vertically. The chamber was filled with an egg suspension, and when the eggs began to settle, their paths of fall were recorded by a camera lucida. The focus of the microscope was previously adjusted to the level at ⅕ of the depth of the chamber (Smoluchowski's layer) and only the eggs which came into sharp focus were selected for measurement. Two points were recorded to determine the path of fall; the electric current was then sent horizontally (i.e., at right angles to the path of fall) and the new path was traced. The deviation of the new path from the extrapolation of the initial path (with no current) corresponds to the cataphoretic movement. The interval of observation was measured by counting the beats of a metronome which was beating once in half a second. The potential drop within the chamber was measured by platinum poles inserted into the chamber through the glass wall by using a voltmeter with a high internal resistance (10,000 ohms, which is about 10 times the resistance of the chamber filled with sea water). The cataphoretic potentials were calculated by the formula $u = DH\zeta/4\pi\eta$, assuming that the dielectric constant of sea water is 80.

In order to minimize daily fluctuations in technique, readings were, as a rule, taken in three adjacent pH values on one day and different combinations of three pH's were studied each day. The data were later handled collectively according to the pH values.

ANTHOCIDARIS CRASSISPINA

The question, whether or not the surface potential is affected by fertilization, has been taken up by several investigators. Gray (1916) examined the agglutination of unfertilized and fertilized eggs of *Sphaerechinus* by $CeCl_3$ and came to the conclusion that fertilized eggs are carrying a more negative potential than unfertilized eggs, basing his conclusion on the fact that a more concentrated solution of cerium cations was required to agglutinate fertilized eggs than unfertilized ones. Runnström's observation (1929, p. 229) tends to support this conclusion. Vlès and Nouel in sea-urchin eggs (1922) and Fauré-Fremiet in the egg of *Sabellaria alveolata* (1924, p. 291) made observations on the behavior of these eggs in an electric field and arrived at the diametrically opposite conclusion, while Szent-Györgyi (1921) contended that sea-urchin eggs bore no charge.

However, some of the above investigations are based on indirect evidence, while the others are not quite free from technical dangers. Therefore, it was considered worth while to re-examine this point by a more direct technique. As a result, in the present paper, not only were the potentials on unfertilized and fertilized eggs compared in normal sea water, but the pH-potential curves of both were constructed through a wider range of the pH-scale. *Anthocidaris crassispina* was selected as one material. In the measurements of the fertilized eggs, the fertilization membrane was left intact. The perivitelline space of this species is very small, which is technically advantageous.

Data are summarized in Table I and graphically represented in Figure 1. (1) The potentials on both unfertilized and fertilized eggs of *Anthocidaris* cannot be reversed in sign as far down as pH 2.0. (2) The maxima of both curves are found in the neighborhood of pH 7.0. (3) Though a statistical separation of the potentials on unfertilized and fertilized eggs at corresponding pH's is impossible, because of the wide fluctuation of the readings, the fact that the average values of the potentials on the fertilized eggs are invariably more positive than those of the unfertilized can be taken as a strong indication that the former are really more positive. Needless to say, this is not due to an increase in the viscosity of the medium by the addition of the seminal fluid, since the eggs are later washed thoroughly. (4) The pH-potential curve for the unfertilized eggs is more zig-zag than that for the fertilized eggs, especially between pH 6 and 7. A repetition of the measurement in the following season (Series II of Table I) gave similar results which were incorporated in the final calculation.

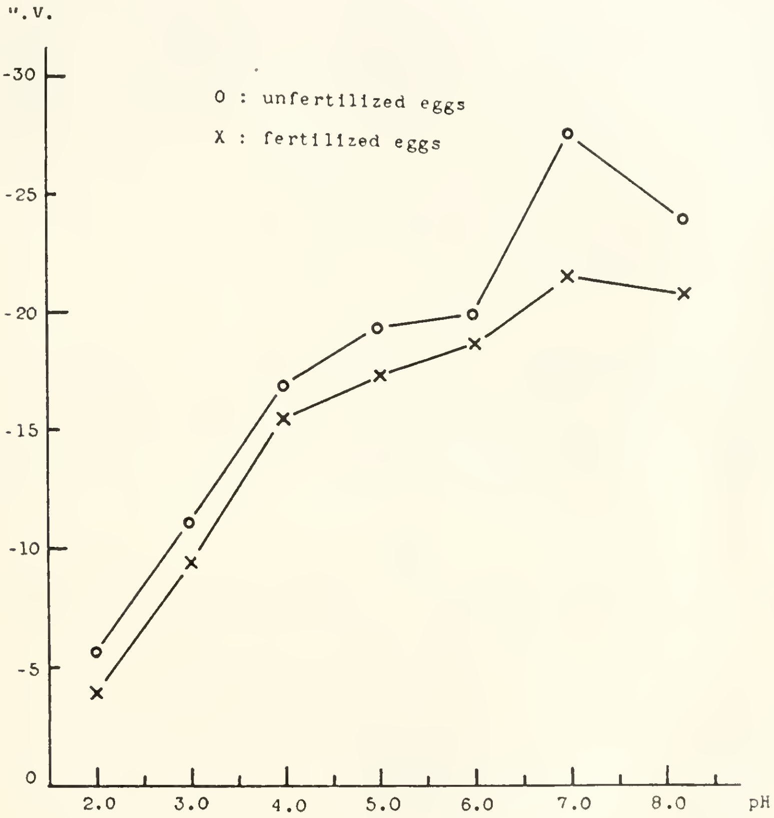

FIGURE 1. Anthocidaris crassispina.

PSEUDOCENTROTUS DEPRESSUS

In order to test the generality of the *Anthocidaris* findings, another sea-urchin *Pseudocentrotus depressus* was studied. However, in this case, attention was paid

TABLE I

Potentials on the egg surface of Anthocidaris crassispina *in millivolts with standard errors*

Medium	Unfertilized eggs without jelly			Fertilized eggs
	Series I	Series II	I and II combined	
Sea water	−24.9±0.78	−23.2±0.38	−23.9±0.34	−20.8±0.79
pH 7	−28.5±0.62	−26.3±0.57	−27.4±0.46	−21.5±0.58
pH 6	−19.9±0.71	−19.7±0.45	−19.9±0.35	−18.6±0.46
pH 5	−19.9±0.71	−18.5±0.43	−19.3±0.38	−17.2±0.61
pH 4	−13.3±0.62	−16.8±0.39		−15.5±0.47
pH 3	−11.0±0.59			−9.4±0.48
pH 2	−5.6±0.90			−4.0±0.81
	Unfertilized eggs with jelly			
Sea water	−24.1±0.41			

chiefly to the unfertilized surface. pH intervals were taken to apparent 0.5 units of the indicators used. Data are summarized in Table II and Figure 2. As is evident from the figure, the general trend of the curve is very much the same as that of the two previous ones, with no reversal of the sign even in the most acidic medium (pH 2) and with a maximum slightly on the alkaline side of neutrality.

TABLE II

Potentials on the egg surface of Pseudocentrotus depressus *in millivolts with standard errors*
Unfertilized eggs without jelly

Medium	Potentials	Indicators
Sea water	−25.0±0.62	Phenol red
pH 8.0	−24.4±0.55	
pH 7.5	−27.0±0.69	
pH 7.0	−26.6±0.57	
pH 6.5	−24.5±0.53	Brom cresol purple
pH 6.0	−23.6±0.60	
pH 5.5	−22.0±0.44	
pH 5.0	−20.1±0.53	Brom cresol green
pH 4.5	−20.4±0.46	
pH 4.0	−16.8±0.43	Brom phenol blue
pH 3.5	−17.0±0.47	
pH 3.0	−13.8±0.55	
pH 2.5	−12.1±0.40	Meta cresol purple
pH 2.0	−9.1±0.59	
	Fertilized eggs with jelly	
Sea water	−28.5±0.71	

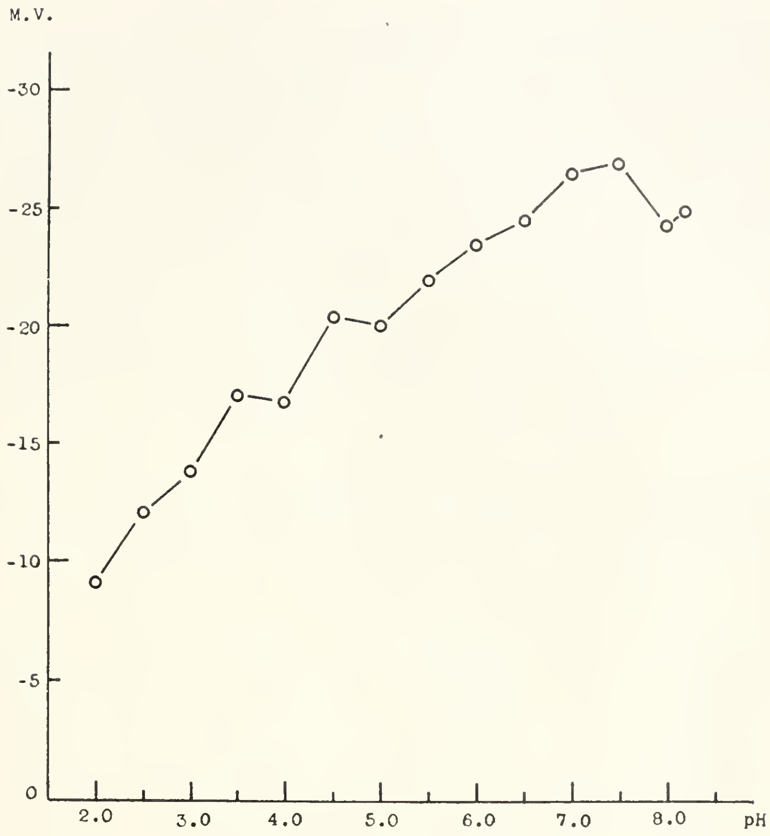

FIGURE 2. Pseudocentrotus depressus.

DISCUSSION

In the first paper of this series, it was reported that the potentials on the unfertilized and fertilized (with the fertilization membrane) eggs of *Arbacia punctulata* were − 30.3 and − 28.7 millivolts respectively and the potential on the unfertilized eggs in a suspension of dead sperm was − 26.7 millivolts. Comparing these figures with the sperm potential and its change under the influence of the egg water of the same species studied by Mudd, Mudd and Keltch (1929) : the potential they found on the sperm was − 22.0 millivolts in sea water and "an increase in negative charge by 13 per cent followed on addition of the egg water." This means that the potential on the sperm in the egg water is − 24.9 millivolts. Now Mudd's experiment and the author's are reciprocal to each other. While the sperm potential which is − 22.0 millivolts changes to − 24.9 millivolts in the presence of the egg proteins, the egg potential which is − 30.3 millivolts at the beginning changes to − 26.7 millivolts in the presence of the sperm proteins. It is highly probable that the mixing of two kinds of proteins will shift the resultant potential somewhere in between the two extremities. This is particularly likely because both the seminal fluid in the author's experiment and the egg water used by Mudd et al. are extremely low in concentration. If a protein is added in high concentration, the potential will coincide with that of a pure protein.

The above postulate acquires a stronger support when the potential changes of other forms are considered. Mudd et al. studied not only the sperm potential of the sea-urchin (*Arbacia punctulata*) but also those of a starfish (*Asterias forbesii*) and a sand-dollar (*Echinarachnuis parma*) and further investigated their changes in egg waters in various combinations. Fortunately, the potentials of the eggs of these species have been measured by the author (Dan, 1934). As is shown in Table III, a sperm potential in egg water either falls between the potentials of the pure sperm and of the egg or coincides with one of them within 1 millivolt. In Mudd's data, the fact stands out as peculiar that the sea-urchin egg water has a specific power to cause a striking increase in the negative potential of any kind of sperm (see Table III, column 3) but this is now easily understandable because the egg protein of *Arbacia* has an exceptionally high negativity.

TABLE III

Comparison of egg potentials, sperm potentials and sperm potentials in egg water. As egg potential, the potential on unfertilized eggs without jelly is used. The figures in parentheses are the percentage changes in negativity of sperm potentials under the influence of egg waters.

Egg potential Sperm potential	Echinarachnius ca. −20.0 m.v.	Arbacia −30.3 m.v.	Asterias −19.9 m.v.
Echinarachnius −16.3 m.v.	−19.6 m.v. (+20%)	−21.7 m.v. (+33%)	−16.3 m.v. (0%)
Arbacia −22.0 m.v.	−21.6 m.v. (− 2%)	−24.9 m.v. (+13%)	−18.9 m.v. (−14%)
Asterias −18.1 m.v.	−17.7 m.v. (− 2%)	−21.7 m.v. (+20%)	−19.2 m.v. (+ 6%)

The present experiment was started with the above view in mind and it was hoped that the difference in the natures of the two proteins (if they are proteins) might be brought up more clearly by constructing pH-potential curves in a wider range. As a result, in the *Anthocidaris*-series, after fertilizing the eggs by the usual technique, excess of sperm fluid was added to secure a complete adsorption and then the eggs were thoroughly washed free from seminal fluid. In spite of this, both curves followed very similar courses except for the fact that the curve for the fertilized eggs consistently stayed below that of the unfertilized eggs.

It may be of some significance to point out in this connection that cholesterol has a very high electric mobility among substances investigated so far. According to Sugawara (1943a, 1943b) the fertilization membrane and its precursor of sea-urchin eggs are dissolved by proteolytic enzymes. This does not necessarily mean that the fertilization membrane and its precursor are made up solely of proteins. They are probably a compound of proteins and lipids and it is not impossible to imagine that one of the constituents predominates in the cataphoretic behavior. Further elucidation is greatly desired.

Concerning the course of the pH-potential curves, the absence of the isoelectric point and the existence of a maximum in the vicinity of pH 7 are worth noticing. Even though nothing definite can be based on the data at hand, these two points may be more or less connected with the high salt concentration of the medium, because cases are known in which the addition of salts either shifts the isoelectric point to the more acid side (Linderstrøm-Lang and Kodama, 1925; Sørensen and Sladek, 1929; Fauré-Fremiet and Nichita, 1927; Haffner, 1922) or brings the maximum around neutrality (Winslow, Falk and Caulfield, 1923). The absence of the isoelectric point here observed is contrary to Ashbel's finding (1931). Her paper states little about the technique of measurement. As far as this author's result is concerned, the pH-potential curves of sea-urchin eggs run quite close to those of *Bacillus cereus* in change of hydrogen ion concentration in concentrated salt solutions studied by Winslow, Falk and Caulfield (1923). The curves resemble each other not only in having no reversal of the sign in the acid range and having maxima near pH 7, but also in a decrease in the migration velocity (or the potential) in the alkaline region. This third similarity will be discussed briefly.

In the above-cited investigation of Winslow, Falk and Caulfield, *Bacillus cereus* loses its negative charge at about pH 10. In *Bacillus cereus* and *Bacillus coli,* the signs of the zeta potentials are reversed around pH 13.5 (Winslow and Shaughnessy, 1924). In the present data on sea-urchin eggs, since the pH scale is not extended so far into the alkaline region, only a downward trend is suspected. On the other hand, however, there is a great deal of indirect evidence from agglutination experiments, indicating that the absolute magnitude of the surface potential of sea-urchin eggs does decrease in the extremely alkaline region. Gray (1916) and Vlès (1924) concordantly found that the eggs agglutinate in a high pH region.

Naturally, a critical analysis is required before we can conclude that in sea-urchin eggs agglutination is really an indication that the absolute magnitude of the potential has decreased as it is in the case of bacteria or blood cells. This problem will be taken up in a later paper.

SUMMARY

1. No isoelectric points were found in unfertilized eggs of *Pseudocentrotus depressus* and in both unfertilized and fertilized eggs (with fertilization membrane) of *Anthocidaris crassispina* when the pH was changed down to 2 in sea water.

2. In all the above cases, the maxima are found in the neighborhood of neutrality. Consequently, in natural sea water (pH, 8.2), the absolute magnitude of the potential is below the highest level.

3. Fertilized eggs carry a less negative potential than unfertilized eggs.

4. The data are discussed in connection with the sperm potentials and their changes in egg water reported by Mudd et al.

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ELECTROKINETIC STUDIES OF MARINE OVA. VI. THE EFFECT OF SALTS ON THE ZETA POTENTIAL OF THE EGGS OF STRONGYLOCENTROTUS PULCHERRIMUS

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In the third paper of this series (Dan, 1936a), the zeta potential of the eggs of *Arbacia punctulata* was measured in NaCl and $CaCl_2$, both isotonic to sea water, and also in mixtures of the two in various ratios. From that study, the conclusion was drawn that the unfertilized surface of the *Arbacia* egg is ordinarily covered by a calcium compound of some sort which, however, dissolves away in the absence of calcium ions. This was based on three facts. (1) In higher calcium concentrations (more than 1/100 M of Ca in the isotonic mixture), the negative potential decreases, roughly corresponding to the calcium concentration, and this change is reversible. This is typical of the ionic effect of divalent cations and it shows that, within this range, the egg surface remains inert. (2) On the contrary, in lower calcium concentrations (less than 1/1000 M Ca), instead of the increase in negativity which is to be expected from the standpoint of the ionic effect, it decreases again and this decrease is irreversible. This is interpreted as due to the permanent loss of the calcium compound. (3) When *Arbacia* eggs are fragmented by centrifugal force into a lighter and a heavier part, the lighter part acquires a potential characteristic of a surface devoid of the calcium compound while the heavier part has a potential identical with that of the compound. This shows that the calcium compound forms a discrete membrane which ruptures on the fragmentation of the cell and is ultimately thrown down toward the heavy pole (Dan, 1936b).

In the first part of the present paper, the same sort of experiment is repeated with the present material and in the second part, the effect of cerium chloride is examined.

MATERIAL AND METHOD

Eggs of the sea-urchin, *Strongylocentrotus pulcherrimus*, were used throughout. Unless specified, the jelly was always removed before the cataphoretic measurements. The method of removing the jelly and the method of measuring the zeta potential are the same as were used in the preceding paper (Dan, 1947).

SODIUM-CALCIUM MIXTURES

The data are given in Table 1 and Figure 1. In the region of higher calcium concentrations, as in the case of *Arbacia*, the absolute magnitude of the negative potential decreases as the divalent calcium ions increase and this change in potential is perfectly reversible. The parallelism between the *Strongylocentrotus*-curve and the *Arbacia*-curve in this region is particularly striking (see Fig. 1). This means that the potential change under consideration is a typical ionic effect due to calcium ions. On the contrary, the two curves diverge in the region of lower calcium concentrations. While the *Arbacia*-curve comes down in dilutions beyond 1/1000 M calcium in mixture, the *Strongylocentrotus*-curve goes sharply up, and this change is reversible.

TABLE 1

The zeta potentials of the unfertilized eggs of Strongylocentrotus pulcherrimus *under various conditions. The values are given in millivolts with standard errors.*

With jelly	-37.3 ± 0.70
Without jelly	-33.9 ± 0.50
Without jelly, 4 hours in NaCl, measured in sea water	-33.9 ± 0.53
Jelly not removed, 3 hours in urea, measured in sea water	-32.9 ± 0.60
Jelly removed by acid, 4 hours in urea, measured in sea water	-33.0 ± 0.50
Heat-killed in NaCl	-26.8 ± 0.44
1/2 M NaCl (intact cells)	-39.7 ± 0.67
NaCl + 1/100 M $CaCl_2$	-33.4 ± 0.64
NaCl + 1/10 M $CaCl_2$	-28.9 ± 0.63
NaCl + 1/5 M $CaCl_2$	-22.7 ± 0.67
1/3 M $CaCl_2$	-17.5 ± 0.40

If the line of argument used for the case of *Arbacia* eggs is pursued further, the above result must be interpreted as indicating that either the *Strongylocentrotus* eggs do not react with calcium ions or the covering layer of the calcium compound of this species is not dissolved away in pure NaCl. The author inclines to adopt the latter view. To some extent, this is supported by the fact that *Strongylocentrotus* eggs are more resistant to cytolysis in NaCl than other species. Of course, this result should not be taken to mean that the covering layer of *Strongylocentrotus* eggs cannot be dissolved at all under any circumstance. The peptization of this compound may occur in some other solutions or even in ½ M NaCl if a different treatment is used. In the present experiment, for the change of medium, the following procedure was selected as standard. To 20 cc. of the egg suspension in a beaker, 200 cc. of ½ M NaCl was gently added and the suspension gently stirred to insure complete mixing. After the eggs had settled, as much as possible of the supernatent solution was siphoned off and fresh NaCl solution was gently added and stirred as before. This washing process was repeated from four to five times. Consequently, the eggs were in the NaCl solution for 30 to 40 minutes before measurement began.

In order to test other possibilities, in one series the time factor was changed. After the usual washings, the eggs were left in NaCl so that the total sojourn of the eggs in this solution was 4 hours. In spite of this the potential did not change (Table 1). In another series, isotonic urea solution was tried and this series was further divided into two groups. In one group, unfertilized eggs with jelly were put directly into urea and left there for 3 hours. Needless to say, the jelly was dissolved by the time cataphoretic measurement was begun. In the second group, the jelly was removed by acid and the eggs were left in urea for 4 hours. Still, no change was observed in the potential. The potential was found to change only after the eggs were killed by heat in NaCl. This is undoubtedly due to the effect of the internal granules (see Dan, 1933). Therefore the above result shows that the surface calcium compound of *Strongylocentrotus* (if there is such a compound) is much more stable than that of *Arbacia* eggs. Unfortunately the number of washings was not changed. At any rate, a definite conclusion can be drawn that the egg surface of this species is quite resistant to the absence of calcium ions and, under the experimental conditions here dealt with, it can be looked upon as an inert surface through the entire range of calcium concentrations.

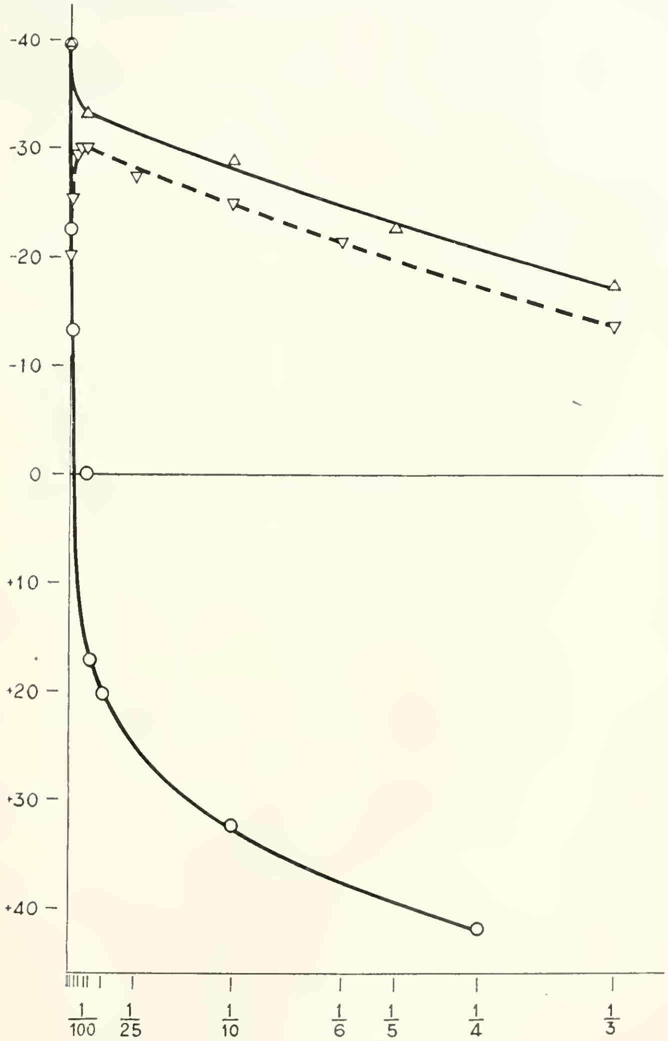

FIGURE 1. The change in the zeta potentials of the unfertilized eggs of *Strongylocentrotus* in mixtures of isotonic NaCl and CaCl$_2$ (△ — △) and of NaCl and CeCl$_3$ (○ — ○). The ratios of the mixtures are expressed by the Ca- and Ce-concentrations respectively which are taken as the abscissae. The ordinates are the potential values. The broken line is the curve for *Arbacia eggs* in Na-Ca mixtures (▽ — ▽). Note the parallelism between the *Arbacia* curve and the *Strongylocentrotus* curve in higher Ca-concentrations and their divergence in the region of low Ca content.

Sodium-cerium Mixture

After examining the effect of calcium, cerium was studied as a representative of the polyvalent ions. The egg jelly was removed and the solutions were changed in the same way as in the calcium series. One-half M NaCl and ¼ M $CeCl_3$ were used either singly or in mixtures. For this purpose, the fact that the surface of *Strongylocentrotus* eggs remains unaffected in pure NaCl is of a great advantage, because it is impossible to mix the cerium solution with sea water on account of the formation of precipitates.

As far as the present material is concerned, the immersion of the eggs in cerium solution is not very harmful as long as the time of sojourn is not too long. On insemination of such eggs after having been returned to sea water, they are fertilizable except for the fact that the fertilization membrane fails to separate from the egg surface (tight membrane eggs).

On the other hand, there are two complications of a physicochemical nature involved in the use of cerium salts. One is the contamination of ceric ions in a preparation of cerous salt. Trivalent cerous ion is rather susceptible to oxidation and it spontaneously changes into tetravalent ceric ion. The former is colorless but the latter is light brown, and this transformation is clearly perceptible if the cerous solution is left in a transparent glass bottle. Unfortunately, this change seems to occur even in the crystals of the former as was revealed by a slight brownish tint of the cerous chloride crystals used by the author. But in the current study, no attempt was made either to obtain colorless crystals or to remove ceric ions, since it was considered that the maintenance of a constant proportion of the two ions would be sufficient for the present purpose. However, an attempt was made to know the ratio of the two by titration after Knorre's method (Knorre, 1897). Ceric ions, 0.0007 M, were found in 0.25 M solution by weight of the crystals; i.e., the ratio was 1 : 357. As a result, in the following paragraphs, the rather loose term "cerium solution" is used instead of specifying the condition precisely.

Table 2

The hydrogen ion concentrations of various mixtures of 1/2 M NaCl and 1/4 M $CeCl_3$. The ratio of a mixture is expressed in Ce concentration after mixing.

Concentrations (M) of Ce in NaCl	1/3000	1/1000	1/100	1/80	1/50	1/4
pH	7.0	6.8	6.2	5.7	5.6	4.7

The other complication is the change in hydrogen ion concentration of cerium solutions. As is well known, among polyvalent cations, it is very difficult to prepare solutions of different concentrations with the same pH, because the ions precipitate. In spite of this, measurements were carried out for a reason which will be explained below. The pH's of the cerium solutions used are given in Table 2.

Results

The result of cataphoretic measurements is given in Table 3 and graphically represented in Figure 1. As is evident, the curve is again typical of an adsorption isotherm, except for the potential value in $^1/_{100}$ M cerium which is obviously off.

In this connection, it must be pointed out that sea-urchin eggs, like any colloidal particles, stick together when the surface potential is abolished. In a $\frac{1}{100}$ M solution the eggs are extremely adhesive. (This point is specially investigated in a following paper.) On account of this, it is quite difficult to take accurate readings. As mentioned above, many eggs clump together in this medium and when large lumps settle, they cause turbulence in the surrounding solution which, in turn, affects the readings. The fact that only a few eggs remain single adds to the difficulty. For such reasons, it seems to be better to determine the iso-electric concentration of cerium solution from the smoothed curve such as is shown in Figure 1.

TABLE 3

The zeta potentials of the unfertilized eggs of Strongylocentrotus pulcherrimus (*without jelly*) *in various mixtures of 1/2 M NaCl and 1/4 M $CeCl_3$, the ratios of the mixtures being expressed in Ce concentrations in the resulting mixtures. The values are given in millivolts with standard errors.*

1/2 M NaCl	−39.7±0.67
NaCl + 1/3000 M $CeCl_3$	−22.7±0.58
NaCl + 1/1000 M $CeCl_3$	−13.3±0.57
NaCl + 1/100 M $CeCl_3$	− 0.4±0.80
NaCl + 1/80 M $CeCl_3$	+17.2±0.49
NaCl + 1/50 M $CeCl_3$	+20.3±0.54
NaCl + 1/10 M $CeCl_3$	+32.3±0.88
1/4 M $CeCl_3$	+41.8±0.80

DISCUSSION

The result reported in the first section of this paper led the author to the conclusion that, contrary to the case of *Arbacia*, the surface of the *Strongylocentrotus* eggs remains inert irrespective of whether calcium ions are present or absent in the surrounding medium. However, this statement signifies at the same time that the change in the zeta potential observed must be brought about by the adsorption of the calcium or cerium ions. In fact, the courses of both calcium- and cerium-curves are quite like any adsorption isotherms, but this situation can be analyzed further.

It is generally understood that the effects of ions on the zeta potential are twofold. One is the effect of ions on the thickness of the diffuse layer (in Gouy's sense) and the other is the adsorption of ions to the surface (Müller, 1933). Fortunately, in such concentrated media as used here, the first proposition is practically negligible and it is permissible to consider solely the second proposition. The next point is that the solutions studied here are not solutions of a single salt but mixtures of two salts. In the Na-Ca series, as the calcium concentration is raised, successively three ions of sodium are replaced by two ions of calcium, and in the Na-Ce series, two ions of sodium are exchanged for one ion of cerium. Besides this, in the cerium series, the hydrogen ion concentration is also changed. But Rona and Michaelis (1919), after studying the adsorption of binary mixtures of salts by charcoal, came to the conclusion that when two salts are present, the more adsorbable ions drive the less adsorbable ions out of the surface so that the net result is very much like the adsorption of a single salt. This simplified picture may be applicable for the present case, since the adsorbability of trivalent cations far exceeds that of monovalent ions for a negatively charged surface.

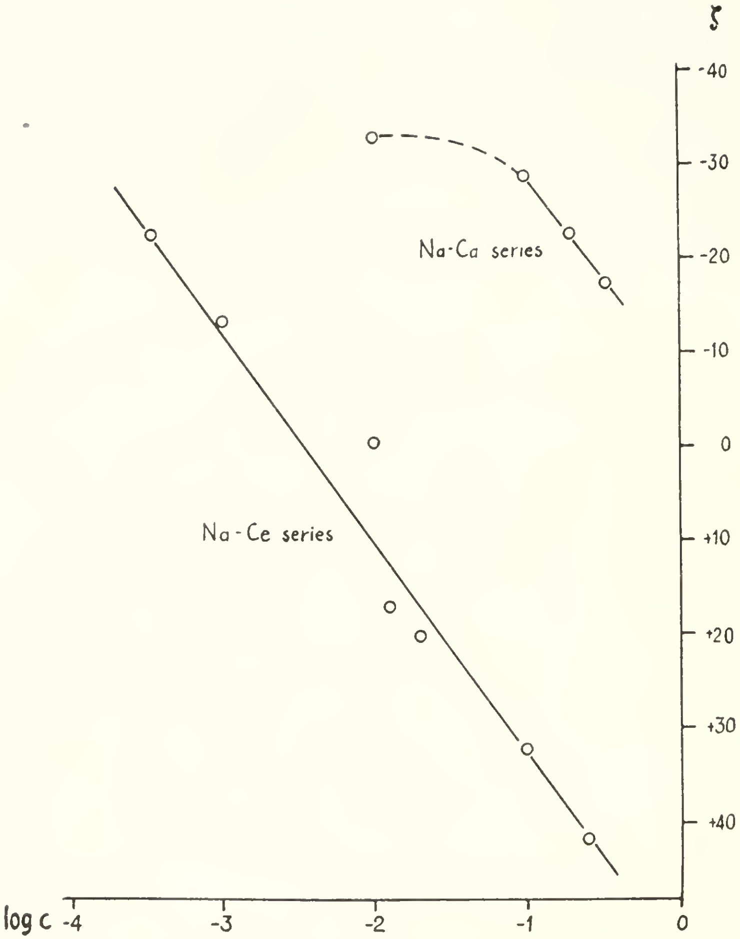

FIGURE 2. The plotting of the potential values against the logarithm of the concentrations. The Ce-series makes a straight line. The Ca-series does so only in the high Ca range.

Allowing this much simplification, the data can be checked by a formula for adsorption. Freundlich's adsorption isotherm will be used for testing, which is

$$a = \alpha \cdot c^{1/n},$$

where a is the quantity adsorbed, c, the concentration, α and n are constants (see Freundlich, 1930, p. 244). As long as this formula holds, plotting of log a against log c ought to give a straight line. For electrokinetics, instead of a, the charge density σ must be used. But since σ is a function of $e\zeta$, in place of log σ, the potential value ζ can be directly plotted (see also Müller, 1933).*

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* $\sigma = \pm \sqrt{\frac{DKI}{2\pi} \Sigma n^0 (e^{-2e\zeta/KT} - 1)}$, where σ is the charge per cm^2.; D, the dielectric constant; K, the Bolzmann's constant, namely, $R/N = 1.37 \times 10^{-16}$; T, the absolute temperature; n^0, the number per cm^3. of ions of valency Z far removed from the surface. In other words, n^0 is connected to the molar concentration c with the relation of $n^0 = c \cdot 606 \times 10^{-20}$. e is the base of natural logarithms; e, the electronic charge.

The curve thus plotted is shown in Figure 2. As is evident, the cerium series makes a straight line (neglecting the value in $\frac{1}{100}$ M). This can be taken as a proof that the change in potential in cerium solutions is due to the adsorption of the cerium cations. A similar plotting for the calcium series is also shown in Figure 2. In this case, although a straight line is obtained for the higher calcium region, it deviates in the lower concentrations. This can probably be interpreted as indicating that in the low calcium region, the effect of sodium ions is no longer negligible because the difference in adsorbability and in the potency in influencing the zeta potential between sodium and calcium is not so great as between sodium and cerium.

The next point of importance is the agglutination of the eggs under the isoelectric condition. However, since this problem will be taken up separately in a following paper, the discussion will be deferred until then.

SUMMARY

1. The electrokinetic potentials of the unfertilized eggs of *Strongylocentrotus pulcherrimus* were measured in mixtures of various ratios of isotonic NaCl and $CaCl_2$ and of NaCl and $CeCl_3$.

2. In the Na-Ca series, as the concentration of Ca-ions in the mixture was raised, the absolute magnitude of the negative potential of the eggs decreased. In the Na-Ce series, with the increase in concentration of Ce-ions, the sign of the zeta potential was reversed.

3. At the isoelectric point, the eggs agglutinated.

4. The course of the potential change can be attributed to the adsorption of the cations.

5. The existence of a covering layer and its dissolution in the absence of Ca-ions such as were seen in *Arbacia* eggs have not been found in *Strongylocentrotus* eggs.

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ELECTROKINETIC STUDIES OF MARINE OVA. VII. RELATION BETWEEN THE ZETA POTENTIAL AND ADHESIVENESS OF THE CELL MEMBRANE OF SEA-URCHIN EGGS

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In the field of colloid chemistry, the electrokinetic potential is a factor of paramount importance for the stabilization of suspensions and emulsions. In a suspension of a single substance the stability is minimum in the isoelectric condition.

Agglutination of living cells on abolishing the surface potential has been observed by many investigators in blood cells (Coulter, 1920, 1922; Northrop and Freund, 1924; Oliver and Barnard, 1925) and bacteria (Northrop and De Kruif, 1922a, 1922b; Eggerth, 1923; Shibley, 1924; Falk, 1928; Mudd, Nugent and Bullock, 1932; Mudd, 1933), and also in some plant cells (Pfeiffer, 1933, 1934). The object of the present paper is to see whether a similar relationship exists between potential and agglutination in such large cells as sea-urchin eggs and to investigate it from the standpoint of surface adhesiveness rather than of agglutination. In the majority of agglutination studies, it is conventional to give the critical potentials for agglutination. The critical potential however fails to give us adequate information about the physical state of the surface in the pre-agglutination states. The author thinks, for this reason, that the study of adhesiveness is better since it leads to a wider viewpoint. Studies of this kind are surprisingly few in the literature, presumably because of the inadequacy of methods of measuring adhesiveness (see Pfeiffer, 1935).

METHOD

In the experiments to be reported, the stream method was adopted. The simple glass apparatus which was constructed is diagrammatically shown in Figure 1. It consists of a series of tubes, each square in cross section but differing in dimension. The end with the largest bore is connected to a large flat reservoir (omitted from the figure). This end serves as the inlet, and at the opposite end, there is an outlet tube provided with a stop-cock. As is evident, for a single run of this outfit, five different fluid current strengths can be obtained, the current strengths being calculated by the amount of liquid flowing through the tube during a definite time interval. By combining a choice of different bores and different pressures, it is easy to get a sufficiently wide range of current strengths. Then by taking the percentages of remaining cells over the initial numbers for different current strengths, a curve can be drawn. This curve will be referred to as the "remainder-curve" in later paragraphs. The area circumscribed by the remainder curve and the two axes indicates the magnitude of the surface adhesiveness characteristic to the conditions under investigation.

The actual procedure of the experiment was as follows: a suspension of eggs of a suitable concentration was prepared, introduced into the inverted apparatus, and the eggs were allowed to settle and lie undisturbed for 10 minutes. (The interval of 10 minutes was arbitarily chosen but this interval was kept constant through all the experiments.) At the end of 10 minutes, the apparatus was reinverted (returned to the normal position), so that the eggs were now hanging from the ceiling, so to speak. Definite areas were then marked out along the midline of the tubes of each dimension, quite close to the outlet end, the total numbers of the cells included within the marked areas were counted, and the solution was allowed to flow for exactly 1 minute (interval arbitrarily chosen). The percentages of cells remaining after 1 minute's flow over the initial numbers in different current strengths give a remainder-curve. The positions of the marked-out areas were selected near the oulet end of each portion to avoid vortices in the flow near the inflow end caused by the narrowing of the tube. The distance from the inflow end necessary for obtaining a laminar flow is a function of the dimension of the tube and the current strength and can be calculated by the Reynolds number often used in hydronamics.

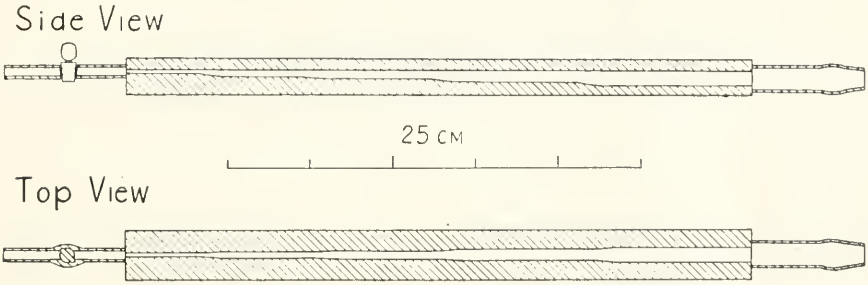

FIGURE 1. A diagram of the apparatus for measuring adhesiveness of the cell membrane. A series of tubes, square in cross section but differing in dimensions, is made through a glass bar (shaded). The large cylindrical tube on the right side is connected to a reservoir by rubber tubing. The small tube on the left side is an outlet and the current can be put on or shut off by the manipulation of the stop-cock. The eggs are made to adhere to the top wall in order to avoid the bumping of the washed-off cells into neighboring cells. It was desired to make the tube of the smallest dimension much longer, but this could not be realized because of a technical obstacle.

CRITICAL CONSIDERATION OF THE METHOD

Current strengths. In the data to be presented, the current strengths are calculated from the cross-sectional areas of the tubes and the amount of solution which flowed out during 1 minute. But, as is known, the current speed is not uniform throughout a cross-section, being maximal in the center and decreasing toward the walls. It is possible to treat this condition mathematically, but considering the other ambiguous terms involved such as 10 minutes' resting or 1 minute's streaming, the calculation was not attempted. Only the precaution was taken of selecting the positions of the marked-out areas along the midline of the top wall.

Turbulences. As was already mentioned, the object of marking out a definite area near the outlet end was to avoid the turbulences in the flow resulting from the narrowing of the tube. But this procedure could not eliminate the secondary turbulences caused by the eggs themselves. In order to remove the errors of this source, care had to be taken to leave a certain distance between two adjacent eggs which could also be calculated. The distance necessary is the greater, the faster the current. As a matter of fact, if an adhering cell is observed by a microscope in a very strong current, the cell is sometimes seen to be vibrating, which will not happen unless it is being washed in a turbulent flow. Although no special attempt was made to remove errors of this kind, they were automatically minimized because of the fact that, in filling the apparatus with an egg suspension, the number of eggs which could get into a smaller tube, where the current speed was greater, was much less than that in a larger tube where the rate of flow was relatively slow.

Glass surface. The inner surfaces of the glass apparatus were polished in order to obtain as uniform a condition as possible and they were frequently cleaned with cleaning solution. But it soon came to the author's notice that the first reading after a cleaning was always higher than the succeeding readings. The readings from the second time on checked very well among each other. This is obviously due to the coating of the glass surfaces with proteins during the first measurement. For this reason, before starting a series of measurements, filling of the apparatus once with an egg suspension was practiced. Between successive measurements, the apparatus was washed with isotonic NaCl solution.

Limit of the method. The maximum current velocity tried in the present investigation was about 2m./sec. When the egg surface was made extremely adhesive by 1/200 M $CeCl_3$, even this flow speed could not wash off any cells. If microscopic observations were made of eggs in a current speed of around 2 m./sec., the eggs were seen to be deformed under the pressure exerted by the current. If the tearing force was further increased, finally the main bodies of the cells were torn off, leaving small portions of cytoplasm still adhering to the glass wall. This is not due to the failure of the membrane adhesiveness to resist the pressure but to the yielding of the membrane tension and the cohering force of the protoplasm to the force applied. This sets a limit to the method.

RESULTS

Hydrogen ion concentrations and adhesiveness. During the work reported in a previous paper (Dan, 1947a), it was noted that when the concentration of hydrogen ions of the medium was increased, the sea-urchin eggs stuck together in various degrees roughly proportional to the concentration of hydrogen ions. In the literature, observations on the agglutination of sea-urchin eggs when an acid is added to sea water are frequently met with (Gray, 1916; Runnström, 1929; Vlès, 1924).

The present experiment was performed on the unfertilized eggs of *Anthocidaris crassispina* from which the jelly had previously been removed. The pH values taken were 8.2 (natural sea water), 6.9, 4.7 and 2.7, salt corrections being made by Clark's table. The cataphoretic potentials at these pH's are − 23.0, − 26.3, − 18.5 and − 11.0 millivolts respectively (Dan, 1947a). The reasons for selecting these pH values are that the first is the natural medium, the second is the medium in which the absolute magnitude of the potential is the largest, the third corresponds to a point which is just above the critical potential for flocculation of the cells and the last is a very acidic medium. The most acidic medium investigated in the previous work (pH 2.0) was intentionally avoided for a reason which will be discussed below.

One of the typical results of the adhesiveness measurements is shown in Table 1 A and B and Figures 2 A and B. It is to be noticed that although variation does exist in the adhesiveness of different batches, the general feature is very consistent.

TABLE 1

The remainder percentages in media of different pH's

A	Stream method										Gravity method
Medium	%	cm./sec.	%	cm./sec.	%	cm./sec.	%	cm./sec.	%	cm./sec.	%
Sea water pH 8.2 (23.0 m.v.)	60.0	0.40	66.7	0.65	13.0	1.28	0	2.00	0*	8.00*	45.8
	49.1	0.40	37.5	0.65	16.7	1.28	3.8	2.00	0	8.00	67.0
Sea water pH 4.7 (−18.5 m.v.)	100.0*	0.41	100.0	0.68*	100.0*	1.33*	100.0*	2.08*	100.0*	8.33*	100.0
	100.0	0.58	100.0*	0.95*	100.0	1.87	100.0*	2.92*	100.0	11.53	100.0
	100.0*	3.74*	100.0*	6.19*	100.0	12.13	70.1	18.96	4.5	75.83	—
	100.0	3.89	100.0	6.43	83.8	12.60	45.3	19.69	0	78.75	100.0
	98.7	4.07	93.1	6.74	79.6	13.20	49.3	20.63	6.3	82.50	100.0
Sea water pH 2.7 (−11.0 m.v.)	—	—	100.0	6.80	98.6	13.33	93.7	20.83	81.5	83.33	100.0
	100.0	7.61	100.0	12.59	98.6	24.67	93.7	38.54	81.5	154.16	—

B	Stream method										Gravity method
Medium	%	cm./sec.	%	cm./sec.	%	cm./sec.	%	cm./sec.	%	cm./sec.	%
Sea water pH 8.2 (−23.0 m.v.)	94.7	0.35	88.3	0.58	67.1	1.13	44.3	1.79	9.5	7.08	7.6
	77.7	0.56	75.5	0.92	56.1	1.80	41.0	2.81	4.8	11.25	—
	100.0	0.33	98.1	0.54	84.9	1.07	73.3	1.67	3.7	6.67	4.9
Sea water pH 6.9 (−26.3 m.v.)	100.0*	0.33*	100.0*	0.55*	100.0*	1.08*	68.4	1.69	5.7	6.75	2.0
	100.0*	0.56*	100.0*	0.92*	100.0	1.80	65.3	2.81	2.9	11.25	—
	—	—	99.1*	0.54*	100.0*	1.07*	72.2	1.67	1.9	6.67	1.6
	—	—	99.1	0.95	98.1	1.86	54.6	2.91	1.9	11.67	—
Sea water pH 4.7 (−18.5 m.v.)	100.0*	0.37*	100.0*	0.61*	100.0*	1.20*	100.0*	1.87*	97.4	7.50	100.0
	100.0*	1.90*	100.0	3.13	100.0	6.13	100.0	9.59	57.9	38.33	—
	100.0*	2.06*	100.0*	3.40*	100.0	6.67	94.5	10.42	36.7	41.67	100.0
	100.0*	3.96*	100.0*	6.53*	100.0	12.79	87.3	20.01	3.3	80.01	—
Sea water pH 2.7 (−11.0 m.v.)	100.0	4.04	98.6	6.66	100.0	13.05	89.7	20.42	83.3	81.67	100.0
	—	—	97.2	11.22	100.0	21.98	89.7	34.39	73.3	147.51	—

*, omitted from figure. {, one lot of eggs exposed to two different current strengths.

There are unmistakable differences between the adhesiveness in pH 2.7 and pH 4.7 and again between that in pH 4.7 and in the two higher pH values. But the difference in adhesiveness between the two higher pH values is hardly perceptible. This is caused by the fact that when the absolute magnitude of the potential increases well above the critical potential, the cells become so non-adherent that the present

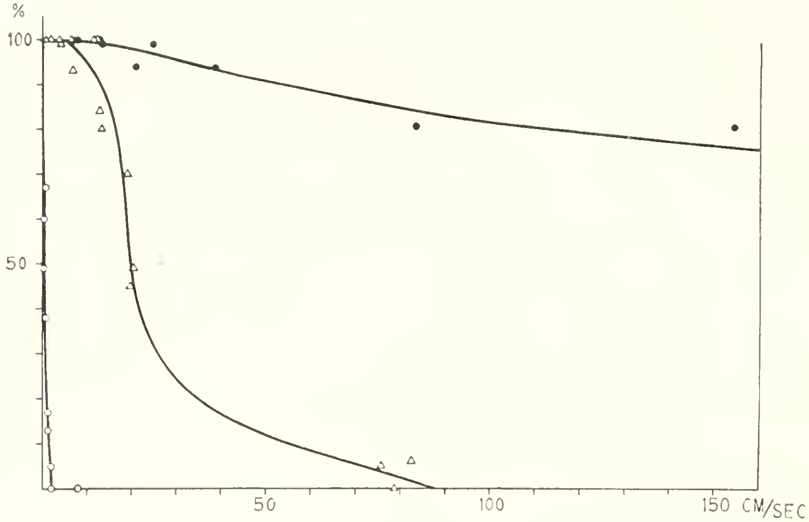

FIGURE 2 A. The remainder-curves of *Anthocidaris* eggs in sea water of pH 8.2 (normal sea water) (○ — ○), pH 4.7 (△ — △) and pH 2.7 (● — ●). The ordinates are the percentages of the remaining cells, and the abscissae are the current strengths. The area circumscribed by a remainder-curve and the two axes is taken as the index of adhesiveness.

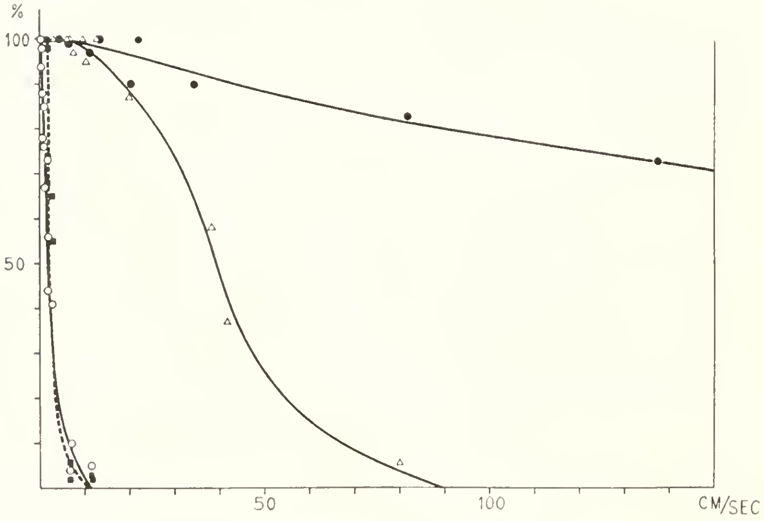

FIGURE 2 B. Another set of *Anthocidaris* remainder-curves in sea water of pH 8.2 (○ — ○), pH 6.9 (■ — ■), pH 4.7 (△ — △) and pH 2.7 (● — ●), showing the reproducibility of the results among different batches. Note also that the adhesiveness in pH 8.2 and that in 6.9 are nearly the same although they can be well differentiated by the gravity method (see Table I B).

technique is too crude to show the difference. Fortunately, however, the difference can be caught by the gravity method. On inverting the container, the number of the cells which fall is greater for pH 6.9 than for pH 8.2 on many occasions (see Table 1 B). In pH 6, the inversion of the tube fails to tear off any eggs from the wall, which indicates that at pH 6, the adhesiveness suddenly increases. It is extremely interesting to remember here that the absolute magnitude of the surface potential in *Anthocidaris* eggs suddenly decreases between pH 7 and pH 6.

It was pointed out previously that the change in cataphoretic potential caused by the change in hydrogen ion concentration is always perfectly reversible. The change in adhesiveness is also reversible. An example is given in Table 2 and Figure 3. The curves for before and after the treatment with the medium of pH 4.7 overlap perfectly. One batch of eggs was met with which became unusually adhesive in the medium of pH 4.7, but even in that case, the reversibility was perfect.

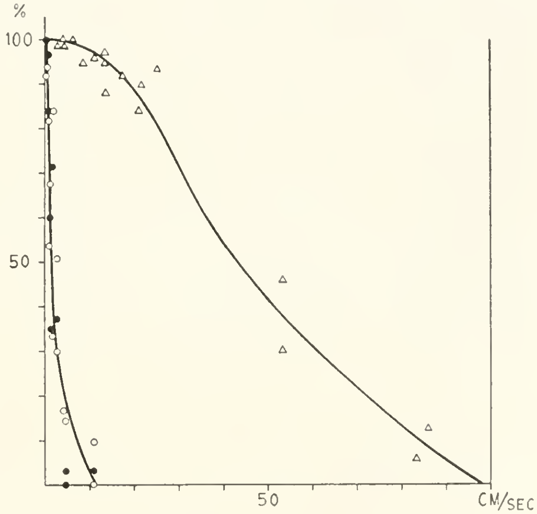

FIGURE 3. A set of records showing the perfect reversibility in the adhesiveness of the cell membrane of *Anthocidaris* eggs. ○ — ○, the remainder-curve in sea water of pH 8.2. △ — △, that in sea water of pH 4.7. ● — ●, that in sea water of pH 8.2 after the treatment with acidified sea water of pH 4.7.

Cerium ions and adhesiveness. The results of the cataphoretic measurements of a Na-Ce series were taken from the preceding paper (Dan, 1947b). These cataphoretic measurements were not made under exactly the same conditions as the present adhesiveness measurements, but they are believed to be comparable.

An example of the adhesiveness measurements in a Na-Ce series is given in Table 3 and Figure 4. This figure is self-explanatory. When the cerium concentration becomes higher, i.e., as the absolute magnitude of the negative potential decreases, the remainder curves become higher and higher and after passing 1/200 M $CeCl_3$, which is nearly the isoelectric concentration, the curves come down again. A comparison of the cataphoretic values with the adhesiveness values is given in

TABLE 2

Medium	%	cm./sec.	%	cm./sec.	%	cm./sec.	%	cm./sec.	%	cm./sec.
Sea water pH 8.2 (−23.0 m.v.)	94.0*	0.08*	91.9	0.14	98.8*	0.27*	81.7*	0.42*	33.3	1.67
	94.0*	0.24*	90.0*	0.39*	95.0*	0.77*	67.5	1.20	14.3	4.79
	94.0	0.55	81.8	0.90	84.0	1.77	50.8	2.76	9.5	11.04
	100.0*	0.21*	100.0*	0.34	84.3*	0.67*	53.7	1.04	16.7	4.17
	100.0*	0.53*	96.2*	0.88	78.1*	1.73*	29.9	2.71	0	10.83
Sea water pH 4.7 (−18.5 m.v.)	100.0*	0.55*	100.0*	0.90*	100.0*	1.77*	98.5	2.76	95.8	11.04
	100.0*	0.85*	100.0*	1.41*	98.3*	2.77*	98.5	4.32	91.7	17.29
	100.0*	2.63*	93.2*	4.35*	96.6*	8.53*	97.1	13.33	45.8	53.33
	100.0*	4.24*	93.2*	7.01*	96.6*	13.73*	89.7	21.46	12.5	85.83
	100.0*	1.23*	100.0*	2.04*	100.0	4.00	100.0	6.25	93.3	25.00
	100.0*	2.63*	100.0*	4.36*	94.7	7.53	87.8	13.33	30.0	53.33
	91.7*	4.12*	96.3*	6.80*	94.7	13.33	83.8	20.83	6.7	83.33
Sea water pH 8.2 (−23.0 m.v.)	90.2*	0.24*	94.4*	0.39*	84.2	0.77	35.0	1.20	0	4.79
	100.0*	0.23*	100.0	0.37	82.1*	0.73*	60.0	1.15	3.1	4.58
	98.2*	0.55*	96.8	0.90	71.4	1.77	37.1	2.76	3.1	11.04

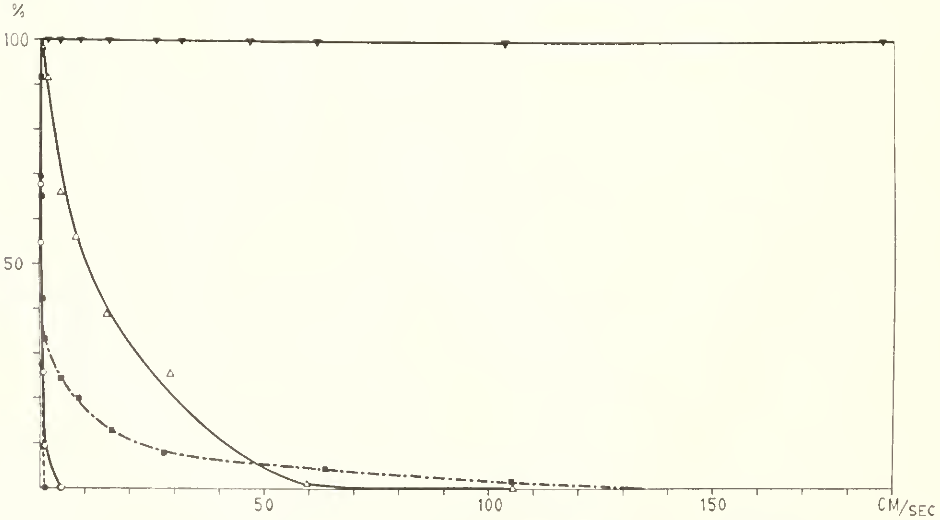

FIGURE 4. The remainder-curves of *Strongylocentrotus* eggs in 1/2 M NaCl (○ − ○), 1/3000 M CeCl$_3$ + NaCl (△ − △), 1/200 M CeCl$_3$ + NaCl (▼ − ▼), 1/50 M CeCl$_3$ + NaCl (■ − ■) and in 1/10 M CeCl$_3$ + NaCl (● − ●).

TABLE 3

Medium	%	cm./sec.	%	cm./sec.	%	cm./sec.	%	cm./sec.	%	cm./sec.
1/2 M NaCl (−39.7 m.v.)	67.7	0.24	54.7	0.39	5.7	0.77	9.4	1.20	0	4.79
1/3000 M $CeCl_3$ in NaCl	97.7	0.57	91.2	1.86	66.0	4.61	56.0	8.13	38.9	15.06
(−22.8 m.v.)	25.5	29.19	0.9	59.55	0	105.42				
1/200 M $CeCl_3$ in NaCl	100.0	1.79	100.0	4.56	100.0	9.11	100.0	15.57	100.0	25.89
(+4.0 m.v.)	100.0	31.53	100.0	46.88	100.0	61.67	100.0	103.54	100.0	187.50
1/50 M $CeCl_3$ in NaCl	91.7	0.24	65.4	0.40	42.2	0.76	33.3	1.22	24.4	4.69
(+21.4 m.v.)	19.9	8.69	12.8	15.91	8.0	27.71	4.5	63.75	1.7	105.00
1/10 M $CeCl_3$ in NaCl (+33.9 m.v.)	69.5	0.23	28.0	0.38	9.2	0.74	0	1.15	0	4.61

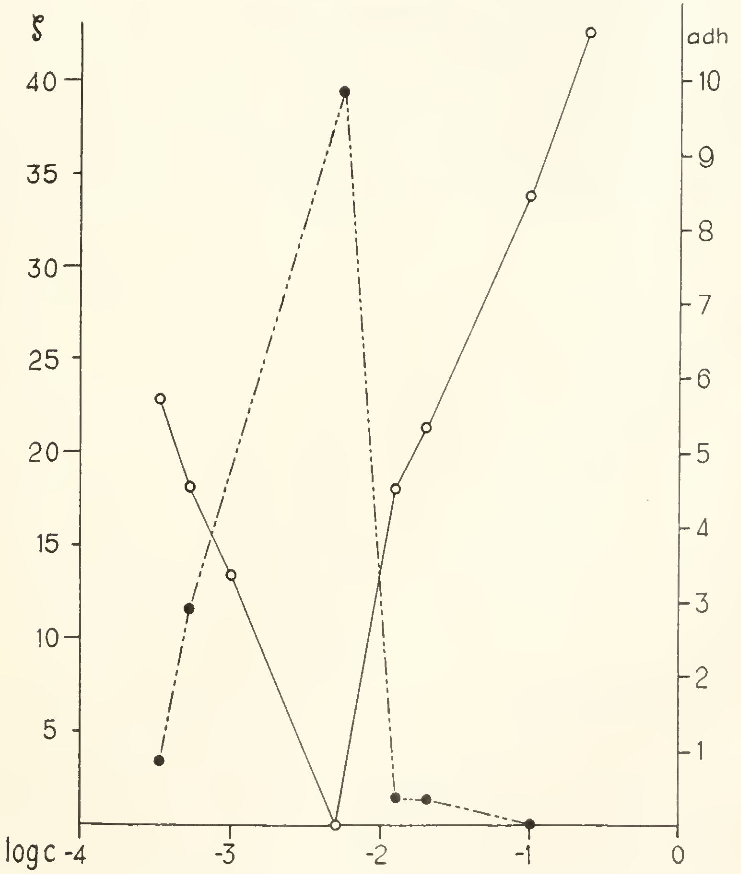

FIGURE 5. Inverse relation between the absolute magnitude of the zeta potential (○ — ○) and the adhesiveness (● —··— ●) as plotted against log *c* of cerium ions.

Table 4 and is represented graphically in Figure 5. Inverse correlation between the two properties is unmistakable. In this case, considering the crudeness of the technique, the adhesiveness values were roughly obtained by cutting out the plotted areas and weighing them instead of measuring the areas by a planimeter. The ratios of the adhesiveness values are in the order given in Table 4: e.g., 1 : 23.5 : 80.5 : ⟩⟩⟩ 273.6 : 10.2 ; 9.3 : 0.7.

TABLE 4

Conc. of Ce-ions in mol.	O(NaCl)	1/3000	1/2000	1/1000	1/200	1/80	1/50	1/10	1/4
Zeta potentials in m.v.	−39.7	−22.8*	−18.1*	−13.4	+4.0*	+18.1	+21.4	+33.9	+42.6
Ratios of adhesiveness values	1.0	23.5	80.5	—	273.6	10.2	9.3	0.7	—

* Potential values found by intrapolation.

SPECIFIC EFFECT OF DIFFERENT IONS ON ADHESIVENESS

If the catophoretic potential is the only factor involved in determining the adhesiveness of the cell membrane, the eggs should have the same adhesiveness at the same potential, no matter by which ions the change is brought about. In order to test this, 1/3 M $CaCl_2$, 1/2000 M and 1/80 M $CeCl_3$ in NaCl were selected and the adhesiveness in these solutions was tested. In all three solutions, the absolute magnitude of the potential is approximately 18 millivolts. The adhesiveness data are given in Table 5 and Figure 6. The figure shows that the above supposition is not true. Although the remainder curves in 1/3 M $CaCl_2$ and 1/80 M $CeCl_3$ follow a more or less similar course, that in 1/2000 M $CeCl_3$ runs decidedly higher. Of

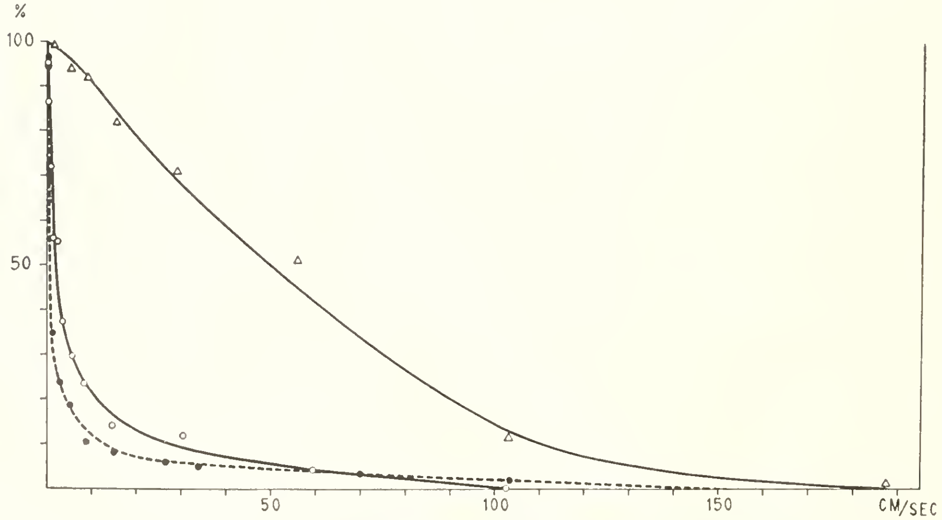

FIGURE 6. Comparison of the membrane adhesiveness of *Strongylocentrotus* eggs in three different media, in each of which the absolute magnitude of the cataphoretic potential of the egg surface is about 18 millivolts. ○ — ○, 1/3 M $CaCl_2$; △ — △, 1/2000 M $CeCl_3$ + NaCl and ● · · · · · ●, 1/80 M $CeCl_3$ + NaCl.

course, there is still room for the argument that since 1/2000 M cerium corresponds to the steepest part of the concentration-potential curve (see Dan, 1947b, Fig. 1), the greater adhesiveness here obtained may be due to a slight deviation of the cerium concentration to the more concentrated side. But a study of the acid series definitely negates this possibility. In a preliminary measurement, at pH 3 where the zeta potential of *Strongylocentrotus* eggs is − 19.6 millivolts, the adhesiveness turns out to be much higher than that in 1/2000 M $CeCl_3$.

Table 5

Medium	%	cm./sec.	%	cm./sec.	%	cm./sec.	%	cm./sec.	%	cm./sec.
1/3 M $CaCl_2$	95.1	0.26	86.5	0.44	71.9	0.81	55.9	1.33	55.4	2.39
(−17.5 m.v.)	37.4	3.58	29.5	5.58	23.3	8.25	14.0	14.60	12.0	30.42
	4.1	59.37	0	102.50						
1/2000 M $CaCl_3$	99.3	1.74	93.9	5.40	91.9	9.20	81.8	15.58	70.9	29.22
(−18.1 m.v.)	51.0	56.01	11.5	103.15	1.1	187.50				
1/80 M $CeCl_3$	94.3	0.25	96.3	0.41	68.5	0.77	34.6	1.28	23.3	2.92
(+17.2 m.v.)	18.5	5.10	10.4	8.80	8.1	15.01	6.1	26.53	5.0	33.75
	3.3	70.21	1.8	103.33						

The above finding is not surprising considering the fact that Northrop and De Kruif (1922a) came across the same sort of phenomenon. They conclude that within 0.01 to 0.1 N of a salt solution, the potential is a primary factor for adhesiveness, but above 0.1 N, the specific effect of individual ions begins to modify the general issue.

Another complication to be remembered is a biological factor. Reports can be found in the literature to the effect that some cells normally have no detectable potential yet are not sticky at all (Mudd, 1933). Among marine ova, *Cerebratulus* eggs belong to this class (Dan, 1934). Besides this, adhesiveness measurements show that immature eggs with germinal vesicles are stickier than ripe ones, with no difference in their potentials. In bacteriology, this secondary modification of the potential-adhesiveness relation is attributed to the state of hydration of the surface. At present, no evidence is available whether or not such a situation exists among marine ova.

At any rate, it can be concluded that so far as sea-urchin eggs are concerned, the cataphoretic potential is a predominant factor in determining stickiness in the majority of the cases, and that only under certain circumstances can biological factors and ionic conditions modify the general trend to a lesser extent.

Discussion

In the preceding paragraphs, evidences have been put forward to show that the adhesiveness of the cell membrane of sea-urchin eggs is primarily determined by the electrical condition of the membrane.

When the zeta potential is shifted, the adhesiveness also changes. Therefore, it is evident that referring only to the critical potentials of flocculation is not a satisfactory method. This shortcoming can be supplemented by the present technique.

However, for the sake of comparison, if the critical potential of *Strongylocentrotus* eggs is suggested, it must lie in the neighborhood of ± 16 millivolts, because in the cerium series, − 13 millivolts is decidedly below the critical point and − 22 and + 21 millivolts are above it, while in the acid series, − 19 millivolts is just above the critical point. This figure of 16 millivolts agrees very well with the other data obtained in a variety of materials.

From the standpoint of cell physiology, two points of particular interest will be commented upon. When the correlation between the abolition of the surface potential and the agglutination of cells was established beyond any dispute in bacteria and erythrocytes, it naturally led investigators to attempt to discover the same relation in leucocytes. However, the careful investigations of Fauré-Fremiet (1927a, 1927b, 1928) and Fenn (1922) revealed the fact that the situation is either very complicated or the correlation fails entirely to hold in the leucocytes of both vertebrates and invertebrates. This fact may be taken as very instructive. As is well known, bacteria and erythrocytes are comparatively inert cells, and as such, are predominantly fitted for the analysis of purely physico-chemical factors. Attempts for further advancements along this path might very likely have been attended with more success if they had been directed to egg cells rather than to leucocytes. The present work will offer a good example to bring out the contrast of the two materials.

The last question is how far the cataphoretic potential is connected with vital activity. In the present stage of our knowledge, no unanimity has been reached among investigators. Many of them even negate a possible correlation between the two. Yet, some positive evidences are accumulating. In the bacteriological field, it is often suggested that the virulence of pathogenic bacteria (Rosenow, 1933a, 1933b, 1934; Rosenow and Jensen, 1933) and the nitrogen fixing power of soil bacteria (Tittsler, Lisse and Ferguson, 1932) are correlated with the surface potential.

As for sea-urchin eggs, the data are too scanty to warrant a definite conclusion. But it is interesting to point out that Schechter (1937), in his study on the endurance of fertilizability of *Arbacia* eggs in mixtures of NaCl and $CaCl_2$ in various ratios, found that the eggs remained fertilizable longest in straight NaCl. This means at least in this case, that the longest viability is associated with the highest magnitude of the zeta potential. (In *Arbacia,* the dissolution of the calcium compound complicates the situation, but so far as the underlying surface is concerned, this statement holds. See Dan, 1936, Table II.)

This interdependence of high negativity and longevity of the eggs has an interesting connection with the electrokinetic conditions prevailing on the egg surface in a normal environment. In *Arbacia*, the potential on the internal granules is ca. − 10 millivolts, that of the protoplasmic surface is ca. − 20 millivolts, that of the calcium compound is ca. − 30 millivolts (Dan, 1936) and finally the potential of the jelly is ca. − 35 millivolts (Dan, 1933). In other words, the zeta potentials of the enveloping layers become higher in absolute magnitude in the order from the interior to the exterior. Among the data so far collected by the author, there is not a single exception to this arrangement (Dan, 1934). A priori thinking, where there is a barrier of a high zeta potential, more energy will be required for charged substances to pass through it, for substances carrying a charge the same in sign as that of the barrier will be repulsed at the boundary, while substances with the opposite sign will be tightly adsorbed. Thus preventing an unnecessary exchange of substances with the environment, this condition may be effective in the protection of the cell interior. This is particularly significant for egg cells which have enough stored nutrient materials to live for a while independent of their surroundings. If we are allowed to go one step further in imagination, this fact may have something to do with the unexpectedly high values in the potentials of egg cells. But until further researches are made, this shielding effect by the high potential of the enveloping layer is simply offered as a suggestion.

Summary

1. A simple apparatus is described for measuring the adhesiveness of the cell membranes of sea-urchin eggs by the stream method.

2. By constructing a curve of the percentages of remaining cells in different current strengths (remainder curve), adhesiveness is expressed by the area circumscribed by the curve and the two axes.

3. In *Anthocidaris* eggs, when the zeta potential is changed by hydrogen ions, the adhesiveness changes correlatively and the shifts in the potential and the adhesiveness are both perfectly reversible.

4. In *Strongylocentrotus pulcherrimus*, both the potential and the adhesiveness change pari pasu when cerium ions are added to the medium. Adhesiveness is maximum at the isoelectric point.

5. Specific effects of individual ions play a secondary role in determining the membrane adhesiveness.

6. Related phenomena are discussed.

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THE DEVELOPMENT OF THE SPIRACULAR CARTILAGES OF THE SPINY DOGFISH, ACANTHIAS VULGARIS (SQUALUS ACANTHIAS)*

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The spiracular cartilages of Selachii differ in their number, shape and development among different forms. Concerning the morphological significance of these elements, there are two different views. The first is that of Huxley (1876) who considers the spiracular cartilage of Heterodontus (Cestracion) to be the separate otic process. Concerning this cartilage, he gives the following statement: "This small cartilaginous plate, which is connected only by ligament with the periotic cartilage above and with the quadrate below, answers to the otic process of the Frog's suspensorium."

Parker (1879) differentiates between the spiracular cartilages of sharks and skates by the following statement: "As a rule, the cartilage developed in the spiracular operculum in the shark is a ray (or rays); in the skate it is part of the body of the arch." Then he adds: "As to Cestracion, I quite agree with him (referring to Huxley) that the spiracular cartilage is the separate otic process, and it is worthy of notice that whilst the spiracular cartilages of the sharks are mere rays, yet they are chondrified detachments of the thin edge of the tissue that fills the primary fold in front of the first cleft."

So while he considers the spiracular cartilages of sharks to be mere rays, yet he agrees with Huxley in considering the spiracular cartilage of Heterodontus, which is also a shark, to be the separate otic process.

Edgeworth (1925) states that the spiracular cartilage of both Scyllium and Heterodontus is formed as a procartilaginous upgrowth of the hind end of the palatoquadrate which subsequently separates and chondrifies. From this he concludes that this cartilage is homologous with the otic process.

In dealing with the skull of Torpedo, De Beer (1926) describes a single spiracular cartilage which is connected with the posterior part of the palatoquadrate by dense tissue, and he is inclined to believe that it represents the otic process.

The otic process of the amphistylic Notidanidae is described by Goodrich (1909) to articulate with the post-orbital process of the aditutory capsule and to pass outside and above the hyomandibular nerve.

The spiracular cartilage does not show these relations which are typical of an otic process. As a matter of fact, it lies far behind and much below the post-orbital process and it is separated from the latter structure by the large levator maxillae superioris muscle. The fact that it lies posterior and somewhat mesial to that muscle shows, as Allis (1914) points out, that it cannot be a part of the otic process.

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* Part of a thesis approved for the degree of Doctor of Philosophy in the University of London.

Against this view also is the fact that whereas there is a single spiracular cartilage in such forms as Heterodontus and Scyllium there may be two of these cartilages as in Acanthias or even three as in Centrophorus. The presence of two or more spiracular cartilages in some Selachians is by no means in favour of Huxley's interpretation.

The other view concerning the spiracular cartilages of Selachii is that of Gegenbaur (1872), who considers these cartilages to be modified mandibular rays.

In supporting this view, Holmgren and Stensio (1936) state that the spiracular cartilages must be interpreted as branchial rays of the mandibular arch. They think that their similarity to the branchial rays is perhaps most obvious in Scymnus, where they are represented by two flat, rod-like cartilaginous plates parallel to each other.

The development of the spiracular cartilages in the spiny dogfish, *Acanthias vulgaris,* favours Gegenbaur's view to a great extent and it shows thoroughly well that these structures are mandibular rays. They are developed in relation to the anterior spiracular wall to which they give support and they have always been found to have the same relation to the mandibular arch as the ordinary branchial rays to their corresponding arches.

They resemble the ordinary branchial rays, not only by their general appearance, both being slender, rod-like structures, but also by the fact that they chondrify at a later period than the mandibular arch. This is quite the same in the case of the branchial rays, the chondrification of which always takes place after that of their corresponding arches.

It has been observed that in a 39 mm. embryo of *Acanthias vulgaris,* where both the palatoquadrate and Meckel's cartilage are present as chondrified elements, the spiracular cartilages have not yet appeared as such. At this stage only the outer spiracular cartilage is present (Fig. 1*A, o.sp.c.*). It is in the form of an elongated mesenchymatous band which stands, more or less, vertically above the posterior end of the palatoquadrate. This band is well-defined and it stains deeper than the surrounding connective tissue from which it is, therefore, easily detectable. It is curved in such a way that its outer surface is convex and its inner one is concave. Its ventral end fits closely on the dorsal surface of the palatoquadrate, nearer to the outside than to the inside. Inner to this spiracular cartilage, is to be seen in the section, the spiracular pouch which is extending, more or less, obliquely and which is connected with the pharynx by an elongated, narrow tube. It opens to the outside in a more posterior section.

The spiracular cartilages were then studied in a 45 mm. embryo, the cartilaginous skeleton of which has been stained with methylene blue by Van Wijhe's method (1902) and micro-dissected. At this stage, not only the outer spiracular cartilage is present, but also the inner one. But they 'are not present as true cartilaginous structures since they have not become stained with the methylene blue. The latter stain, as Van Wijhe pointed out, differentiates not only between cartilage and other tissues, but also between cartilage and procartilage. Although the spiracular cartilages did not become stained with the methylene blue as other parts of the cranium in which chondrification proceeded, they are more rigid than the surrounding tissues and could be easily shown in the dissection.

They are present at this stage as two slender procartilaginous rods which form nearly right angles with the posterior part of the palatoquadrate (Fig. 2, *i.sp.c.* and *o.sp.c.*). They are somewhat curved towards the posterior end of the animal and their ventral ends are in contact with the upper surface of the palatoquadrate.

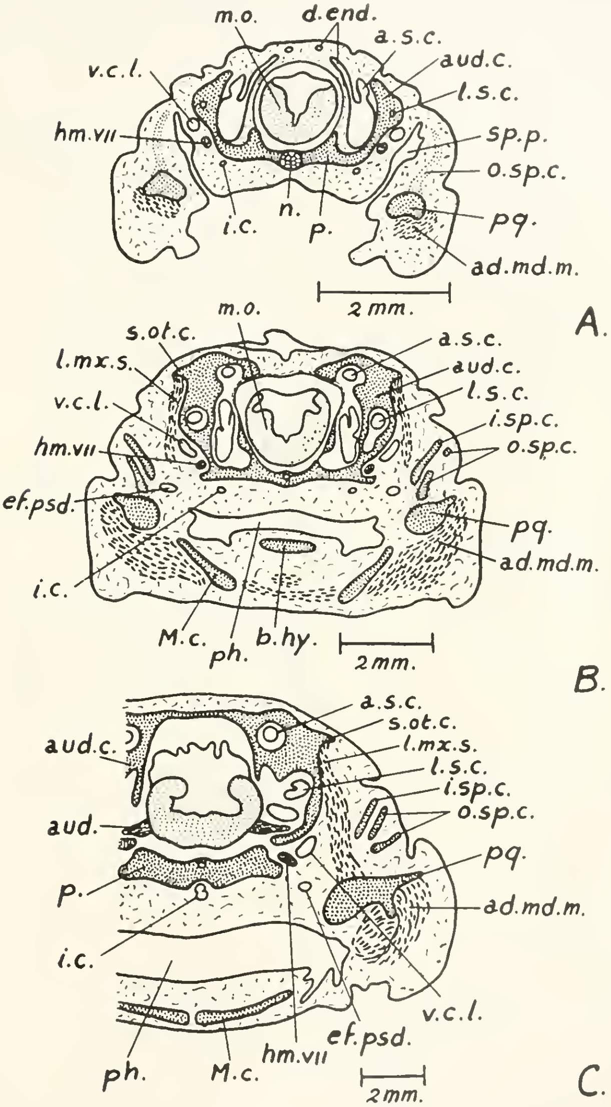
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FIGURE 1. Transverse sections in the head region of *Acanthias vulgaris* showing the spiracular cartilages of: *A,* 39 mm.; *B,* 64 mm. and *C,* 110 mm. embryos.

These ventral ends are broader than the dorsal ones which are pointed. The outer spiracular cartilage, which, as stated above, appears earlier than the inner one is more elongated than the latter element. Their relation to the palatoquadrate, is here strikingly similar to that of the hyal rays to the hyomandibular and ceratohyal, as well as to that of the branchial rays to both the epibranchials and ceratobranchials.

Mori (1924), in describing a 44 mm. embryo of Acanthias, states that there is a single spiracular cartilage which is oval in shape and which has its longer axis parallel to that of the palatoquadrate. This description is not correct as it has been found that the spiracular cartilages are always, in Acanthias, rod-like structures which stand at right angles to the palatoquadrate.

Although Sewertzoff (1899) describes also a single spiracular cartilage in his 50 mm. stage, yet his description of this single element is in accordance with that of the 45 mm. embryo given above. This cartilage is rod-like and is at right angles to the palatoquadrate.

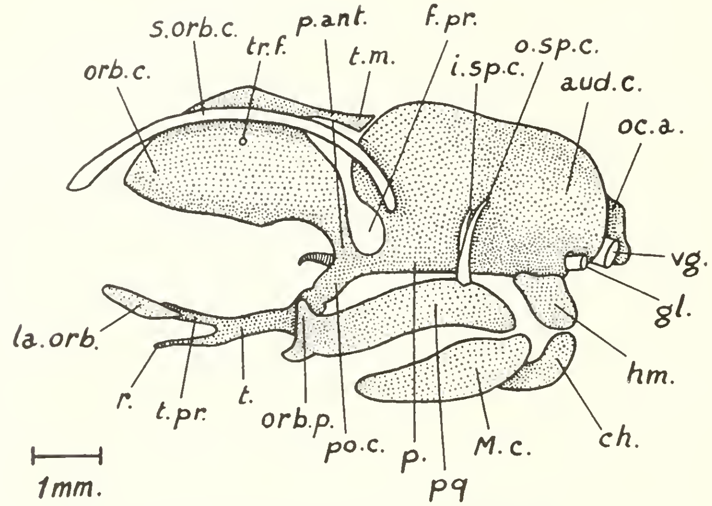

FIGURE 2. The chondrocranium of 45 mm. embryo of *Acanthias vulgaris* stained with methylene blue and micro-dissected to show the relation of the spiracular cartilages to the palatoquadrate.

In the following stage, which is a 64 mm. embryo, the two spiracular cartilages have chondrified and they still retain their approximation to the palatoquadrate (Fig. 1*B*). The outer spiracular cartilage is thicker than the inner and its base is closer to the palatoquadrate. The inner has grown more dorsally than in the previous stage. The ventral ends of both of them are surrounded by mesenchymatous tissue which extends ventrally to lie in contact with the dorsal surface of the palatoquadrate. Dorsal to the spiracular cartilages and lateral to the auditory capsule, is shown, in the section, the most posterior part of the levator maxillae superioris muscle (*l.mx.s.*). It is attached dorsally to the supra-otic crest of the auditory capsule which is, here, not yet well-developed.

In a later stage (102 mm. embryo) the development of the spiracular cartilages proceeded further by their growth in the dorsal direction, particularly the inner one which now exceeded the outer in length. Its dorsal end lies now opposite the upper surface of the lateral semicircular canal (Fig. 3*A*). The relation of the two spiracular cartilages to the palatoquadrate is the same as before and again the outer lying closer to it than the inner. They are somewhat facing backwards and they lie right at the back of the levator maxillae superioris muscle.

Whereas these cartilages are quite simple rods in the previous stage, their structure is here rather interesting. The outer spiracular cartilage has a bifurcate dorsal end, the outer of the two branches is somewhat broadened to form a small lamella while the inner one is slender (Fig. 3*A′*). The dorsal end of the inner spiracular cartilage is, on the other hand, divided into two branches which are nearly of the same thickness. The tips of the four branches lie, in the figure, on a curved line which represents the margin of the anterior spiracular wall.

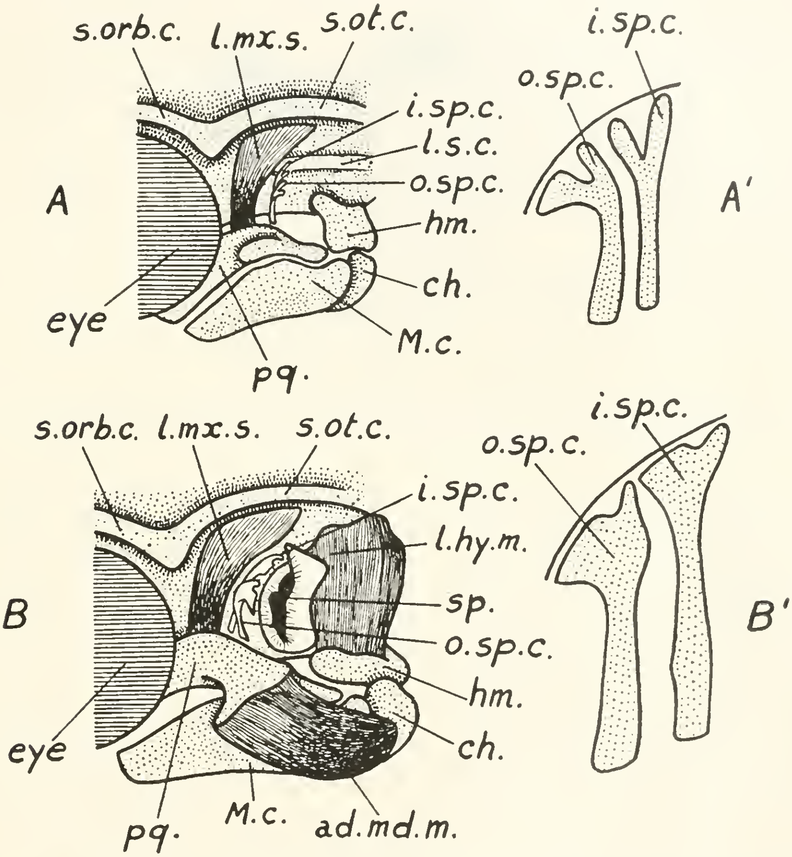

FIGURE 3. Lateral and posterior views of the spiracular cartilages of 102 mm. embryo of *Acanthias vulgaris* (*A* and *A′*) ; same of 270 mm. embryo (*B* and *B′*).

The distinct bifurcation of the dorsal ends of the two spiracular cartilages is interesting owing to the fact that they show great resemblance to the branching hyal rays of the same stage. These latter rays are shown in Figure 4 where some of them are simple rods of cartilage while others have bifurcate posterior ends. Great similarity is to be observed on comparing the two spiracular cartilages with these bifurcate hyal rays of the same individual. Each one of the latter structures possesses a simple stalk which lies opposite the ceratohyal and a bifurcate posterior end which lies away from it. The spiracular cartilages are strikingly similar to them and they differ only in extending dorsally instead of posteriorly as these rays extend. This similarity favours the view that they are nothing but mandibular rays formed to support the anterior spiracular wall.

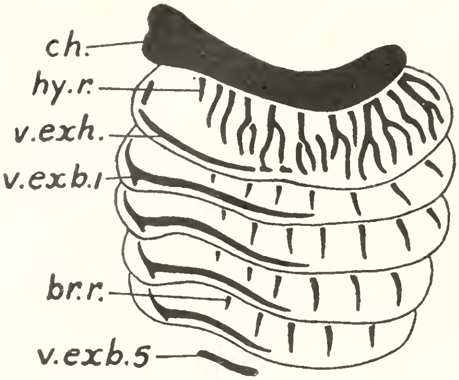

FIGURE 4. Hyal and branchial rays of 102 mm. embryo of *Acanthias vulgaris* stained with methylene blue and micro-dissected.

The spiracular cartilages are also shown to be bifurcate in a still older stage (110 mm. embryo) which has been sectioned. In this latter embryo they are more developed than in the previous stage and they spread over a large area in the spiracular wall. The two branches of the outer spiracular cartilage are shown in the section (Fig. 1*C, o.sp.c.*). Of the inner one, only the outer branch is found in the same section, while the inner branch is to be seen in another section. Inner to them, there is the posterior part of the levator maxillae superioris muscle which extends from the supra-otic crest above to have its ventral insertion on the dorsal surface of the palatoquadrate. The latter cartilage is here well-developed and possesses a prominent ventral process which adds to the efficiency of the attachment of the adductor mandibularis muscle to that cartilage. Inner to the palatoquadrate there is the efferent pseudobranchial artery (*cf. psd.*) which extends from the spiracular pouch to unite with the internal carotid artery (*i.c.*) after piercing its way through the lateral cranial wall.

The bases of the two spiracular cartilages are present in a more posterior section where they lie quite close to the palatoquadrate cartilage, the same as in the previous stages. This section is chosen mainly to show the two branches of the outer spiracular cartilage which are extremely distinct.

Finally the spiracular cartilages have been dissected in a 270 mm. embryo, a stage in which the yolk sac has completely disappeared and the embryo is ready to leave the body of the female fish and lead a free life. They are here broader than before in the transverse direction, so that in a posterior view (Fig. 3*B′*) they appear as two small plates of cartilage instead of being rod-like as in the previous stages. At the same time the bifurcation of their dorsal ends is about to disappear completely. This is brought about by the growth of the two branches of each spiracular cartilage towards each other to fill up the space lying between them. This growth has advanced in this stage to such an extent that only the tips of the original branches are to be observed. As a result, the dorsal part of each spiracular cartilage is much broader than its ventral part and it is present as a somewhat broad, flat lamella carried by the original stalk of each cartilage. The original bifurcation, though nearly completely obscured, is still to be detected at the upper margin of that lamella.

In a lateral view, the spiracular cartilages are strongly curved backwards, this curvature following that of the spiracular pouch (Fig. 3*B*). Their ventral ends are also in contact with the upper surface of the palatoquadrate.

The spiracular cartilages of this stage are quite similar to those of adult Acanthias where they are described by Wells (1917) as two small, flat cartilage bars. The only difference is the complete disappearance of the original bifurcation of their dorsal ends in the case of the adult fish.

The development of the spiracular cartilages in *Acanthias vulgaris,* therefore, supports completely the view expressed by Gegenbaur that these cartilages are simply mandibular rays. Summarizing the facts upon which this conclusion is based, they are as follows:

1. They chondrify at a later period than the mandibular arch which is in complete accordance with the chondrification of the ordinary branchial rays with respect to their arches.
2. They are, particularly at early stages of development, quite simple rods of the same shape as the branchial rays and they articulate with the palatoquadrate in a way which is similar to the articulation of the latter rays with their corresponding arches.
3. The fact that at a certain stage of their development they have bifurcate dorsal ends and that this bifurcation makes them quite identical with the bifurcate hyal rays of the same stage is a good support to that view.
4. At no stage of development whatsoever, have they been found to be in continuity with the palatoquadrate, a fact which is against the idea that they represent the separate otic process.
5. The fact that they lie posterior to the levator maxillae superioris muscle and far away from the post-orbital process shows that they do not occupy the position of a typical otic process.

When we compare the function of the spiracular cartilages, which are supporting the anterior spiracular wall, with that of the branchial rays, a great similarity between both structures is to be observed. The branchial rays are developed in relation with the gill lamellae to which they give support. It is well-known that the spiracular pouch represents the remains of an originally functional gill pouch which existed between the mandibular and hyoid arches and which was comparable with any of the following gill pouches. Such a functional spiracular pouch is described by Watson (1937) in the Acanthodians. It existed between the mandibular and hyoid arches with the result that the latter arch was free and did not function as a suspensorial element. In these latter fishes, not only the palatoquadrate but also

Meckel's cartilage, carries a complete set of mandibular rays. They are developed to support a mandibular operculum which is present in these fishes.

The existence of a functional gill pouch between the mandibular and hyoid arches is, therefore, from the palaeontological point of view, beyond reasonable doubt. When such a functional gill pouch has been reduced into the spiracular pouch during the phylogenetic development of the Selachii, it follows that the rays related to this pouch should also be reduced in number. In the latter fishes, therefore, it is to be considered that the spiracular cartilages are the remains of a complete set of mandibular rays which existed in their ancestoral forms and which used to support a functional gill pouch lying between the mandibular and hyoid arches. Even after this reduction in number took place, the function is still the same and the spiracular cartilages are present to support the walls of the reduced spiracular pouch.

Acknowledgment

I wish here to express my deep gratitude and indebtedness to Professor D. M. S. Watson, Head of the Zoology Department, University College, London, for the great help he offered me while doing this work under his supervision. It is through his advice that the whole work dealing with the development of the chondrocranium of *Acanthias vulgaris* has been satisfactorily completed.

Explanation of Lettering

a.s.c., anterior semicircular canal;
ad.md.m., adductor mandibularis muscle;
aud., auditory nerve;
aud.c., auditory capsule;
b.hy., basihyal;
br.r., branchial rays;
ch., ceratohyal;
d.end., ductus endolymphaticus;
ef.psd., efferent pseudobranchial artery;
f.pr., foramen prooticum;
gl., glossopharyngeal nerve;
hm., hyomandibular cartilage;
hm.VII, hyomandibular branch of facial nerve;
hy.r., hyal rays;
i.c., internal carotid artery;
i.sp.c., inner spiracular cartilage;
l.hy.m., levator hyomandibularis muscle;
l.mx.s., levator maxillae superioris muscle;
l.s.c., lateral semicircular canal;
la.orb., lamina orbitonasalis;
M.c., Meckel's cartilage;
m.o., medulla oblongata;
n., notochord;
o.sp.c., outer spiracular cartilage;
oc.a., occipital arch;

orb.c., orbital cartilage;
orb.p., orbital process of palatoquadrate;
p., parachordal;
p.ant., pila antotica;
ph., pharynx;
po.c., polar cartilage;
pq., palatoquadrate;
r., rostrum;
s.orb.c., supra-orbital cartilage;
s.ot.c., supra-otic crest;
sp., spiracle;
sp.p., spiracular pouch;
t., trabecula;
t.m., taenia marginalis;
t.pr., lateral trabecular process;
tr.f., trochlear foramen;
v.c.l., vena capitis lateralis;
v.exb.1–5, first to fifth ventral extrabranchials;
v.exh., ventral extrahyal;
vg., vagus nerve.

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